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THE GLOMERULAR DEVELOPMENT OF THE VERTEBRATE KIDNEY IN RELATION TO HABITAT

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Some twenty-five species of fishes, belonging to twelve families, are known to possess nearly or completely aglomerular kidneys (Marshall, 1929, and this paper). It has been suggested that this aglomerular condition is related to the peculiar water cycle associated with a marine habitat (Smith, 1930). In fresh water a large quantity of pure water is absorbed probably by way of the oral membranes, and excreted in large part, if not entirely, by the kidneys; whereas, in sea water a relatively small quantity of sea water with its contained salts is absorbed from the gastro-intestinal tract, and a large fraction of the absorbed water is excreted extra-renally. Thus a persistent oliguria relative to fresh-water forms occurs in marine fish, and this oliguria is held to be the cause of the glomerular degeneration.

In this view glomerular development should be related to water excretion in the vertebrates generally, and we wish to present evidence in this paper that such is the case. This evidence takes the form of three arguments: (1), that the protovertebrate kidney was aglomerular and that the glomerulus was evolved as an adaptation to a fresh-water habitat, (2) that in the lower vertebrates the extent of glomerular development is related to the quantity of water normally excreted by the organism and (3) that in the mammals (and possibly to some extent in lower vertebrates) the primitive water-excreting function of the glomerulus has been secondarily diverted to a filtration-reabsorption system designed to excrete waste products without the loss from the body of excessive quantities of water.

The Protovertebrate Kidney

The only information available on the nature of the protovertebrate kidney is that which may be obtained from the embryonic development of the lower vertebrates. There are no essential differences in the development of the pronephros of the cyclostomes, fishes and Amphibia, but we may suppose that the cyclostomes approach most closely to the primitive condition. Of these the developmental history is best known in the case of *Petromyzon* which has been studied by Wheeler (1899), Hatta (1900–01) and Inukai (1929). Additional information on the vertebrate pronephros is given by Felix in Hertwig's Handbuch (1906).

The pronephros of the lamprey at an early stage consists of six tubules on either side, formed from mesoderm and opening by means of nephrostomes into the unsegmented body cavity just behind the branchial region. In a later stage some of the tubules at the cranial and caudal end degenerate while the remainder (according to Inukai from 3 to 6 pairs) develop and function for a considerable time during the larval period. These pronephric tubules communicate with the pericardial cavity, which at about this time has been cut off from the peritoneal cœlom. Hatta regards the segmental duct as being formed by a series of abortive pronephric tubules in about twelve somites lying posterior to the eighth somite. After the formation of paired tubules, further development of the pronephros consists in the lengthening and convolution of the tubules, and the formation of a blood supply and glomerulus.

The blood vessels which supply the pronephros acquire definite form in a comparatively late stage of development. The glomerulus or glomus is lobulated, supplied by at least three branches of the aorta and hangs free in the pericardial cavity.

Hatta comes to the conclusion that in the ancestors of *Petromyzon* the pronephros once extended over all the body segments from the branchial to the cloacal region, the tubules opening to the exterior in each segment. The tubules of the posterior region were later converted into the segmental duct which first opened to the exterior and then into the cloaca.

In teleosts, where several originally distinct pronephric tubules have fused to form the pronephric chamber and a single tubule, the glomerulus is evolved after the separation of the pronephric chamber from the cœlom has taken place. Therefore, the pronephric tubule is aglomerular and unconnected with the cœlom for a considerable time during development (Emery, 1882; Felix, 1906). The glomerulus is here an inner glomerulus and consists of an invagination of a capillary tuft into

the dilated end of the tubule which forms the pronephric chamber. Besides this condition and that observed in *Petromyzon*, there is a third and more usual type of pronephric development where the glomerulus is formed by a more or less complete abstraction of the true body cavity with an outer glomerulus or glomus in a pronephric chamber. Of these three types it would appear that that of the lamprey is the most primitive. Here, the glomerulus is quite distinct, separate from, and formed much later than the pronephric tubules.

That the glomerulus was developed after and secondarily to the pronephric tubules in the early vertebrates is certainly suggested by the above discussion. It is borne out by many other considerations. In invertebrates, nothing resembling a glomerulus occurs, but we find as excretory organs various types of tubules with glandular epithelium. The mesonephros of teleosts is stated to be aglomerular in young embryos (Audigé, 1910). In certain mammals (*e.g.*, rat and mouse) the mesonephros never develops glomeruli (Bremer, 1916). In most vertebrates the mesonephros is usually first formed of segmental tubules connected to the cœlom by nephrostomes. Later the glomerulus develops and we have a tubule containing a typical Malpighian body with its glomerulus, but still connected to the body cavity by a nephrostome, as in the adult Urodeles. However, the tubule in these forms usually loses its connection with the cœlom (Widersheim, 1906). Borcea (1906) states that in the development of the mesonephros of certain elasmobranchs (*e.g., Raia*) the segmental tubules lose their connection with the body cavity before the formation of glomeruli, thus paralleling the development of the pronephros of teleosts.

We may infer, then, that at some period the protovertebrate kidney was aglomerular and that it probably consisted of a series of tubules communicating with the cœlom by means of nephrostomes, the tubules either opening separately to the exterior or opening into a common duct. The tubules extended over a greater portion of the body than is the case with the pronephros at present. The tubules were not simple conduits, but their epithelium was glandular in structure and they may have both reabsorbed substances from the cœlomic fluid passing through them and added to this fluid by secretion. That secretion was a function of the primitive tubules is suggested by the fact that the tubular epithelium is derived from cœlomic epithelium and by the fact that crystals have been found in the lumen of the blind pronephric tubule of the embryo trout before the glomerulus has developed (Nussbaum, 1886.)

The cœlom seems to have been the original excretory organ, being connected with the exterior by means of pores, (*c.f.*, abdominal pores

of cyclostomes and elasmobranchs) or by tubules opening into the cœlom by means of nephrostomes. Felix (1906) says, "als primitivestes Harnorgan haben wir fraglos die Leibeshohle selbst anzusprechen." The excretory function of the primitive cœlomic epithelium is indicated by the connection of both pronephros and mesonephros with its cavity, by the existence of abdominal pores in cyclostomes and elasmobranchs, and by the composition of the cœlomic and pericardial fluids of the latter (Smith, 1929b).

With the development of glomeruli, the nephrostomal connection of the tubule with the cœlom is usually abolished. The nephrostomes draining the cœlom may be secondarily diverted to empty into the venous system (Amphibia), into lymphoid spaces (elasmobranchs) or they may disappear completely.

Ever since Bowman published his original theory of urinary secretion in 1842 the glomerulus has been assigned the rôle of eliminating most of the water of the urine. All subsequent theories have taken this premise as their starting point. When this developmental and functional evidence is coupled with the evolutionary history of the lower vertebrates it appears probable that the glomerulus was evolved in response to the need for an easy means to excrete large quantities of water.

There is much evidence in the geological nature of the strata in which the early fossil vertebrates are found to indicate that these animals were inhabitants of fresh or slightly brackish water (Chamberlain, 1900; Barrell, 1916; O'Connell, 1916; Kiâer, 1924; Geikie, 1903; Woodward, 1900; Grabau, 1921; Hussakof and Bryant, 1918; Patten, 1912; Stromer, 1920). The subject presents many palæontological and geological difficulties and must still be considered a controversial one. According to Barrell (1916) with slight extensions based on the observations of other palæontologists and geologists, it would appear that the Silurian and Devonian ostracoderms and fishes were inhabitants of the continental rivers and fresh-water lakes. From some unknown relatives of these fresh-water forms there were evolved the Devonian elasmobranchs, dipnoans and ganoids and later the Carboniferous Amphibia. The elasmobranch fishes migrated to the sea toward the middle or late Devonian and, though frequently invading fresh water in subsequent times, this sub-class is predominantly marine today. The ganoid fishes may have invaded the sea to some extent in the Devonian, but so far as those forms which were ancestral to the recent teleosts are concerned, the permanent assumption of a marine habitat appears to date more properly from Carboniferous or even Mesozoic times. Judging in part from the historical record and in part from the life habits

of the recent fishes, it would appear that the recent dipnoans, ganoids and many primitive teleosts have had a more or less continuous fresh-water history since the early Palæozoic period, while the recent marine teleosts may be assumed to have lived in pelagic or deep ocean waters only through Mesozoic and Tertiary time.

The composition of the sea in past ages is unknown, but it may be inferred that the salinity in the Devonian period was at least half, if not three-quarters, of what it is at present. It is certain that the salinity of the ocean waters throughout the Tertiary has been great enough to impose upon the marine teleosts the same osmotic restraints in regard to the absorption and excretion of water and salts that characterize this habitat today.

When these separate lines of evidence are brought side by side it is a logical deduction that the glomerulus was evolved in some early Palæozoic chordate to enable the organism to excrete readily the large quantity of water which was absorbed along the osmotic gradient existing between its blood and its fresh-water environment. This glomerulus represented simply an advantageous juxtaposition of the blood-vascular system to the already existing tubular system draining the excretory cœlom.

So long as the organism remained in fresh water (dipnoans, ganoids and teleosts) or in intimate dependence upon it (Amphibia), this excretory arrangement persisted; but with the secondary assumption of a marine habitat (teleosts) where the osmotic gradient was reversed and the water excretion reduced, or with the assumption of terrestrial life in which water conservation became a necessity (arid-living reptiles and birds), the organism no longer needed and could no longer economically use this primitive water-excreting mechanism. There was thus a need to either (*a*) discard or reduce the glomeruli or (*b*) amend their primitive function by adding distally a more efficient mechanism for the reabsorption of water. The first process appears to be occurring in the marine teleosts and in the reptiles. In the mammals and possibly to some extent in the birds, on the other hand, the addition of the loop of Henle has permitted the reabsorption of water against the osmotic pressure of the metabolites in the urine; consequently in these the glomeruli, although still very active as filters, have become secondarily incorporated into a filtration-reabsorption system which permits the excretion of waste products without the excretion of excessive quantities of water.

In substantiation of this thesis we wish to present here a description of the glomerular development in some fresh-water and marine fishes and in a few other vertebrates. No attempt has been made to interpret

the structure of the kidney in larval forms because nothing is known at present about their water excretion or osmotic relationships. The marine cyclostomes and elasmobranchs possess blood which has about the same osmotic pressure as sea water, and such limited knowledge as we have of these animals indicates that the water cycle in them is quite different from what it is in the marine teleosts. Until more information is available on these points we cannot expect to fit these sub-classes into the present hypothesis.

The Glomerular Development in Adult Vertebrates

In examining different animals we have used the following criteria in judging glomerular development:

1. Reduction in the number of glomeruli and presence of aglomerular tubules (here the extreme stage is an aglomerular kidney).
2. Very small size of the glomeruli.
3. Lack of good vascularization in the glomerular tuft, whether due to the presence of excessive amounts of connective tissue or to lack of capillary branching.

When only one of these conditions occurs one cannot draw definite conclusions, but if two or all conditions are present, it seems safe to conclude that glomerular development is poor.

The Higher Fishes.—Our observations have been most extensive in the heterogeneous group of teleostean fishes. We have collected a large series of data here in order to test our hypothesis.

The kidney of teleosts has not been extensively or intensively studied. It appears that wide variations in its structure can occur. What is known at present of the structure of its renal tubule has been summarized in a recent paper (Marshall, 1930). The long paper by Audigé (1910) is the most complete on teleost kidneys. Following Hyrtl (1851), Audigé divides the kidney into an anterior, middle, and posterior kidney. He further states that the posterior kidney is a metanephros, has well developed large glomeruli with branching capillary tufts, and that no aglomerular tubules occur; that the middle kidney has none, few, or many glomeruli, which are small and consist of a single coiled capillary; and that the anterior kidney in most adult forms consists entirely of lymphoid tissue. On this basis one can divide the teleosts into two groups, those with and those without a posterior kidney. We made such a division from all the data we could collect from the literature. Although in general the marine teleosts fell into the group without posterior kidney (poor glomerular development);

and the fresh-water fish into that with posterior kidney (good glomerular development), there were many exceptions. Our subsequent histological study of sections from the kidneys of many teleosts convinced us that a classification on the basis of the examination of sections was much more accurate than the above, and hence we omit this preliminary classification.

We have divided the higher fishes into four groups on the basis of glomerular development as shown from a study of sections of the kidney. These are as follows:

Group I. Kidneys having frequent glomeruli which are medium or large and invariably well vascularized. This group presents extremely good glomerular surface.

Group II. An intermediate group. Kidneys may have frequent glomeruli which are very small, or may have few glomeruli which are small or fair sized. Kidneys never have both frequent and medium sized glomeruli.

Group III. Kidneys having infrequent glomeruli which are small, poorly vascularized and which may show signs of degeneration. This group presents extremely poor glomerular surface.

Group IV. Aglomerular kidneys. In this group are included *Lophius*, where the very few glomeruli present are non-functional in the adult (Grafflin, 1929), and several species described by Guitel (1906) in which a single large glomerulus occurs in the persistent pronephros, but in which the remainder of the kidney (mesonephros) is aglomerular.

There would appear to be little error involved in placing a species into groups I and IV, but some selection is necessary to determine whether a species should go into group II or III and to a less extent as to whether it should go into group I or II. Any questionable case has been put in the intermediate group II, so that it is possible that some species in group II should have been in group I or III.

The following table gives the groups as defined above and the average size of the renal corpuscles in sections of kidney fixed and treated in the same way.[1] The habitat of the species studied is given in

[1] Measurements of the size of the renal corpuscle in fixed sections are undoubtedly too small due to shrinkage. Another objection to measuring glomeruli in sections is the difficulty of always measuring a section cut through the middle. This error will affect the larger glomeruli more than the smaller. Distortion of the shape of a Malpighian body also occurs in sections. This has led us to average the two diameters measured in ten corpuscles and give a single figure as representing the size. Using our measurements only to indicate whether the glomeruli are small, medium or large would seem to be justified in spite of the many unknown factors which must be considered in comparing different animals. We have worked only with adult animals.

TABLE I

Glomerular Development and Habitat of Higher Fishes

Species	Family	Common Name	Habitat	Size of Renal Corpuscle in Micra
Group I				
Protopterus æthiopicus Heckel	Lepidosirenidæ	Lung-fish	F	153
Polypterus senegalus Cuvier	Polypteridæ		F	89
Amia calva Linnaeus	Amiidæ	Bowfin	F	82
Plecostomus plecostomus (Linnaeus)	Loricaridæ		F	73
Cyprinus carpio Linnaeus	Cyprinidæ	Carp	F	82
Carrassius auratus (Linnaeus)	Cyprinidæ	Goldfish	F	62
Catostomus commersonii (Lacépède)	Cyprinidæ	Sucker	F	60
Ameriurus nebulosus (LaSueur)	Siluridæ	Catfish	F	99
Morone americana (Gmelin)	Serranidæ	Silver perch	E	63
Ambloplites rupestris (Rafinesque)	Centrarchidæ	Bass	F	86
Enneacanthus gloriosus (Holbrook)	Centrarchidæ	Sunfish	F	76
Perca flavescens (Mitchill)	Percidæ	Perch	F	102
Salmo gairdneri Richardson	Salmonidæ	Trout	E	60
Salvelinus fontinalis (Mitchill)	Salmonidæ	Trout	E	75
Umbra limi (Kirtland)	Esocidæ	Mud minnow	F	106
Esox lucius Linnaeus	Esocidæ	Pickerel	F	81
Anguilla rostrata LaSueur	Anguillidæ	Eel	E	104
Gymnothorax funebris Ranzani	Murænidæ	Moray	M	112
Myoxocephalus octodecim (Mitchill)	Cottidæ	Sculpin	M	81

TABLE I (*Continued*)

Species	Family	Common Name	Habitat	Size of Renal Corpuscle in Micra
Group II				
Copeina guttata (Steindachner)	Characidæ		F	48
Astanyx sp.	Characidæ		F	50
Centropristis striatus (Linnaeus)	Serranidæ	Sea bass	M	52
Hæmulon album Cuv. and Val.	Hæmulidæ	Grunt	M	34
Hæmulon album Cuv. and Val.	Hæmulidæ	Grunt	M	64
Holocentrus ascensionis (Osbeck)		Squirrel fish	M	39
Gadus callarias Linnaeus	Gadidæ	Cod	M	37
Melanogrammus æglefinus (Linnaeus)	Gadidæ	Haddock	M	38
Hemitripterus americanus Gmelin	Cottidæ	Sea Raven	M	65
Chilomycterus schœpfi (Walbaum)	Diodontidæ	Spiny box fish	M	59
Caranx ruber (Bloch)	Carangidæ	Jack	M	33
Sphyræna barracuda (Shaw)	Sphyrænidæ	Barracuda	M	35
Strongylura raphidoma Ranzani	Sphyrænidæ	Hound fish	M	46
Tautoga onitis (Linnaeus)	Labridæ	Tautog	M	56
Scomber colias Gmelin	Scombridæ	Tinker mackerel	M	48
Cryptacanthodes maculatus Storer	Cryptacanthodidæ	Wrymouth	M	94
Pseudopleuronectes americanus (Wal.)	Pleuronectidæ	Flounder	M	50
Poronotus triacanthus (Peck)	Triglidæ	Butter fish	M	51
Stenotomus chrysops (Linnaeus)	Sparidæ	Porgy	M	51
Chætodipterus faber (Broussonet)	Chætodipteridæ	Spade fish	M	40
Balistes vetula Linnaeus	Balistidæ	Trigger fish	M	41
Sebastes marinus (Linnaeus)	Scorpænidæ	Rose fish	M	44

TABLE I (*Continued*)

Species	Family	Common Name	Habitat	Size of Renal Corpuscle in Micra
Group III				
Argyropelecus hemigymnus Cocco	Stomiatidæ		M	
Chauliodus sloanei B. and S.	Stomiatidæ		M	
Gonostoma bathyphilum (Vaillant)	Stomiatidæ		M	
Gonostoma grande (d?)	Stomiatidæ		M	
Strongylura notatus (Poey)	Belonidæ	Needle fish	M	39
Strongylura sp.	Belonidæ	Bill fish	M	37
Cypselurus heterurus (Rafinesque)	Exocœtidæ	Flying fish	M	30
Teuthis hepatus Linnaeus	Teuthidæ	Brown tang	M	27
Sphæroides maculatus (B. and S.)	Tetradondidæ	Puffer	M	38
Lactophrys bicaudalis (Linnaeus)	Ostrociidæ	Shell fish	M	26
Group IV				
Syngnathus dumerilii (Kaup)	Syngnathidæ	Pipe fish	M	None
Syngnathus acus Linnaeus	Syngnathidæ	Pipe fish	M	"
Siphonostoma sp.	Syngnathidæ	Trumpet fish	M	"
Nerophis lumbriciformis (Willoughby)	Syngnathidæ		M	"
Entelurus anguineus (Kaup)	Syngnathidæ		M	"
Hippocampus brevirostris Storer	Syngnathidæ	Sea horse	M	"
Hippocampus guttulatus Cuvier	Syngnathidæ	Sea horse	M	"
Lepadogaster gouanii Bris. de Ban.	Gobiesocidæ		M	"
Lepadogaster bimaculatus (Pennant)	Gobiesocidæ		M	"
Lepadogaster candolli Risso	Gobiesocidæ		M	"
Lepadogaster macrocephalus (d.?)	Gobiesocidæ		M	"
Chorisochismus dentex (Pallas)	Gobiesocidæ		M	"
Gastrostomus bairdi Gill and Ryder	Saccopharyngidæ		M	"
Pterophryne histrio (Linnaeus)	Antennariidæ	Mouse fish	M	"
Opsanus tau (Linnaeus)	Batrachidæ	Toad fish	M	"
Lophius piscatorius Linnaeus	Lophiidæ	Goose fish	M	"

the third column, *F* indicating fresh water, *M* marine and *E* euryhaline (where the fish can live in fresh or salt water). The evidence for the aglomerular nature of the kidneys of group IV is given by Marshall (1929) and that for placing the first four fish in group III by Nusbaum-Hilarowicz (1923). The kidneys of all the remaining fish have been examined in the course of this study.[2] The microphotographs of typical fields in cross sections of the kidney given at the end of this paper illustrate fairly well the differences between these groups (Plates I, II and III).

The relationship of habitat to glomerular development is brought out forcibly by the extremes represented in groups I and IV.

The fishes of group IV are aglomerular and thus represent the extreme condition which we imagine to result from a very low water excretion, namely, the complete absence of all filtering surface as typified by the glomerulus, and the presence only of tubular tissue of a high, cuboidal and secretory nature. The fish included in this group are all marine, and even related species do not migrate into fresh water except in rare instances which we may believe represent secondary invasions of fresh water. (The Syngnathidæ have representatives in Panama which may occasionally be found in brackish to fresh-water streams). They are all end-products or terminal members in the evolution of specialized Teleostei. The Saccopharyngidæ are abyssal or deep water forms, though occasionally the latter may invade brackish water. The remaining families are littoral or pelagic forms, and though some of them (*Opsanus*) may normally invade brackish waters, none is known to migrate into strictly fresh water.

The fishes of group I represent the primitive condition which we imagine existed in the Palæozoic ganoids which were ancestral to all the Teleostei, marine and otherwise. In these the glomeruli are numerous, large, with very flat capsular epithelium and the capillary tuft is finely divided by branching. Here the filtering surface is obviously extensive. These species are with two exceptions (*Myoxecephalus* and *Gymnothorax*) either exclusively fresh-water or euryhaline forms. *Ameriurus, Myoxocephalus, Gymnothorax* and *Plecostomus* are relatively highly specialized forms; the others are primitive types. The Loricaridæ, Siluridæ, Cyprinidæ and Characidæ belong to the Ostariophysi, which is typically a continental and fresh-water order. *Amia* is a primitive ganoid and *Protopterus* and *Polypterus* represent the two most ancient types of surviving fishes.

[2] The specimens of *Protopterus* and *Polypterus* were collected by H. W. S. in Africa through the favor of the John Simon Guggenheim Memorial Foundation.

In contrasting these groups we note that the members of groups III and IV consist of aglomerular or nearly aglomerular fish, of a generally specialized type, which are exclusively marine; group I consists of well glomerularized fish, of a generally primitive type, which are with few exceptions exclusively fresh-water or which occasionally enter fresh water. It is perhaps significant that all the abyssal marine forms which have been studied fall into groups III or IV while the continental and pelagic forms fall into groups I and II. An abyssal habitat may be taken as evidence of a long marine history. It is to be expected that aglomerular kidneys would more probably occur in fishes which had been marine for a long period of time. The fact that the aglomerular forms are in other respects specialized rather than primitive is in accord with general principles of evolution.

Between the extremes of fresh-water or primitive marine fish, on the one hand, and highly specialized marine fish on the other, it is to be expected that there would occur every degree of glomerular development. That such is the case is shown by the character of the fish which we have placed in the intermediate groups II and III. Group III consists of fish with greatly reduced glomerular development; the glomeruli are small, poorly vascularized and very infrequent. These fish are all marine and specialized forms. They approach quite closely to the members of group IV in general character and in glomerular development. It is possible that among these fish some of the few remaining glomeruli are non-functional.

The members of group II are intermediate in glomerular development and heterogeneous in character. They are mostly marine, and include specialized as well as more generalized forms. It is, of course, in this group that the need of a more quantitative expression of glomerular development is most pressingly felt, but this gap in our knowledge is not the only deficiency; we have no information as to how long these fish have been marine or to what extent they may have migrated from salt to fresh water, or *vice versa,* in the course of teleostean evolution. Without such information a quantitative interpretation of this group is practically impossible.

It may be noted that some instances of secondary invasion of fresh water are clearly evident. Thus in the sub-order Apodes the Saccopharyngidæ, Murænidæ and most of the Anguillidæ are marine; but the common eel, *Anguilla rostrata,* lives in fresh water and only returns to the sea to spawn. It may be inferred, then, that a fresh-water habitat is relatively recent for this species. The deep-sea *Saccopharyngida, Gastrostomus bairdi,* is aglomerular; while *Anguilla* shows the typical

fresh-water development of the glomeruli. It is probable that the fresh-water Percidæ and Centrarchidæ and the brackish-water Pleuronectidæ, Tetradondidæ and Cottidæ represent forms derived from marine stocks and secondarily entering fresh-water.

Until more is learned, both about the physiology of these forms and their evolutionary history, a closer interpretation than that attempted above is clearly impossible.

Amphibia.—A large amount of work has been done on the kidneys of the Amphibia, yet there is no evidence that aglomerular tubules ever occur in this class. The glomeruli appear to be numerous, well vascularized and the capsule has a low epithelium. This is in line with the close ecological dependence of the Amphibia on fresh water.

The largest glomeruli of all animals occur in the Urodeles (Hyrtl, 1863). Steinbach (1927) gives measurements of the size of glomeruli from sections of several species of Amphibia. These vary from 86 to 217 micra. Using our technique, we find for the average size of the glomerulus in *Rana catesbeiana,* 115 micra; *Bufo americanus,* 92 micra; *Plethodon cinereus,* 145 micra; *Siren lacertina,* 211 micra; and *Necturus maculosus,* 308 micra.

There are some Amphibia which burrow and remain underground during the drought season (North Central Australia). Sweet (1907) states that these forms take in large quantities of water by the mouth and skin before estivating and excrete and store it in the urinary bladder. The water is reabsorbed from the bladder into the abdominal cavity during estivation and passed by the nephrostomes (of which these species have an exceptionally large number) into the renal veins; in this way the water is used over and over again. This absorption of water from the urinary bladder is in line with the recent observation of Steen (1929) that, when exposed to dry air, frogs may reabsorb all the water from the urinary bladder. Sweet states that in one of these burrowing species (*Chiroleptes alboguttatus*) the glomeruli are very few in number and remarkably small.

But as a class the amphibians are provided with good glomerular surface. It is difficult to compare the separate species because of the wide variation in the size of the animal and of the size of the glomerulus. We have not attempted any quantitative analysis within this class.

Reptiles.—The glomeruli of reptiles are peculiar in that the center of the glomerular tuft consists of connective tissue with capillaries only on the outside (Regaud and Policard, 1903–04; Cordier, 1928). In addition to this peculiarity, the Malpighian corpuscle is rather small. Bowman (1842) gives the diameter of the glomerulus of the tortoise

as 106 micra, and of the boa constrictor as 63 micra; Hüfner (1866) gives for *Testudo græca* and *Emys europoea,* 110 micra; Regaud and Policard (1903–04) give for the Ophidia, 110 x 90 and 80 x 60 micra; and Hoffman (1890) gives for the Saurians 45 to 52 micra. Zarnik (1910) reports measurement of the glomeruli of the longest tubules of several species of reptiles from macerated preparations, but since they cannot be taken as average values, they are of no assistance to us here.[3] Cordier (1928) reports the average size of the glomerulus in the Chelonians as 50 micra, in the Ophidians as 80 micra and in the Lacertilians as 50 micra. He made careful reconstructions of the glomeruli of these forms and came to the conclusion that the capillary is never an unbranched vessel as stated by Regaud and Policard (1903–04); but the filtering surface of the glomerulus in Chelonians is fairly good and in Ophidians and Lacertilians extremely poor. Cordier clearly recognizes that this difference is related to the elimination of water. He finds from sections that the decrease in filtering surface of the glomerulus of snakes and lizards is not compensated for by increased number, and relates the poor glomerular development of the former to their solid or semi-solid excrement; and the much better development in Chelonians, to their fluid urine.

Table II summarizes our observations on a few species.

TABLE II

Glomeruli of Reptiles

Scientific Name	Common Name	No. Renal Corpuscles	Size in Micra	Vascularity of Tuft
Pseudemys sp.	Slider Terrapin	Frequent	63	Fair
Caretta caretta (Linnaeus)	Sea Turtle	"	91	"
Alligator mississippiensis (Daudin)	Alligator	"	—	"
Boa imperator Daudin	Boa Constrictor	Infrequent	71	Poor
Liopeltis vernalis (Harlan)	Grass Snake	"	59	"
Phrynosoma cornutum (Harlan)	Horned Toad	"	51	"

Two distinct groups of reptiles can be established: one with fair glomerular surface and fluid urine (Chelonia and Crocodilia); the other with very poor glomerular surface and solid or semi-solid urine (Lacertilia and Ophidia). It is in this last group that Regaud and Policard (1903–04) and Zarnik (1910) have found blind diverticula or

[3] As a rule no statement is given as to how the measurements were made (sections, macerated tissue, or fresh material). This probably accounts for many of the wide discrepancies in the figures. The size of the animal from which the kidneys were removed would also be an important factor.

aglomerular tubules. Microphotographs of sections from kidneys of the terrapin, horned toad, and boa constrictor are given at the end of this paper (Plates III and IV).

Birds.—In the bird's kidney we find the beginning of the loop of Henle. Many tubules occur without the loop, however, and these have a short intermediate segment similar to that of reptiles. Many other tubules show transitional stages (Huber, 1917; Feldotto, 1929). The glomeruli of birds' kidneys are stated to be the smallest known; they are, however, no smaller than some occurring in marine teleosts. The capillary loop of the glomerulus is described by some observers as unbranched and by others as only slightly divided. Bowman (1842) states that the "Malpighian vessel is a coiled ampulla"; Hyrtl (1863) says that in spite of the extremely small size of the glomerulus, the vessel is not simple but divides in the smallest glomeruli into 2, and in the largest into 7 or 8 branches. Standfuss (1908) also remarks on the small number of capillary loops in the glomeruli of birds.

Bowman gives the diameter of the glomerulus of the parrot as 59 micra and Hüfner (1866) gives for the dove 44 x 35 micra. Von Mollendorff (1922) has measured the glomeruli of several birds in macerated preparations. He finds average diameters of 48 micra for the pigeon, 38 micra for the ring-sparrow, 28 micra for the house-sparrow, and 24 micra for the finch. We have examined sections of the kidneys of the chicken and pigeon, and find the average diameter of the Malpighian bodies to be 70 micra in the former and 50 micra in the latter. A curious fact about the glomeruli of these birds, which so far as we know has not been noted before, is that the central part of the glomerular tuft resembles the reptilian glomerulus in its lack of capillaries. There is, however, instead of connective tissue a central core of dense syncytial-like tissue. A microphotograph of a section from a chicken kidney is given (Plate IV).

It is obvious that the bird's kidney shows glomerular degeneration, as indicated by the very small size and poor vascularization of the glomeruli, and by the replacement of the central portion of the tuft by syncytial tissue. It is improbable that increased number of glomeruli can offset this reduction in filtering surface.

Mammals.—So far as is known, the tubules of mammalian kidneys are supplied with large, well vascularized glomeruli. Bowman (1842) gives the average size of the glomerulus of a number of mammals ranging from 100 micra in the mouse to 362 micra in the horse. Many subsequent observers give measurements for different species (See Vimtrup, 1928, and v. Mollendorff, 1929). Thus the smallest mammalian glomerulus can be considered large in comparison with those occurring in many marine teleosts, reptiles and birds.

It is in the mammalian kidney that the loop of Henle attains for the first time its full development. This fact is significantly associated with the marked hypertonicity which mammalian urine can exhibit in comparison with the blood plasma (Crane, 1927) and suggests that the glomerular function in these animals has been almost completely modified from its primitive water-excreting function. It is interesting to note that in the primitive *Echidna*, the kidney resembles that of the reptile as much as that of the higher mammals, and that here the glomerular development is stated to be less than in other mammals of the same size (Zarnik, 1910). Much more work, however, will have to be done before mammals can be compared in a quantitative way with other animals or among themselves.

Discussion

The idea that the development of the glomerulus is an adaptation to water excretion by the kidney agrees well with the facts which we have presented.

In the diversified group of higher fishes, we find convincing evidence of the influence of a marine habitat on the development of the glomerulus. In the snakes and lizards as well as the birds, where water conservation is so important that a solid or semi-solid urine is eliminated, the unquestionable reduction in glomerular surface is entirely in accord with what might be predicted.[4] The good glomerular development of the fresh-water fishes and the Amphibia is quite in line with the general thesis. Difficulties arise in attempting to compare different animals with one another in a quantitative way. It is essential to know the number of glomeruli in the kidneys, and also the surface area presented by the capillaries of an average glomerulus. One can obtain the number by accurate counts, but there is no accurate method for obtaining the surface area of a glomerulus and more particularly of the variable capillary tuft. It appears to be generally true that the smaller the glomerulus, the less the capillary tuft is broken up by branching, but the amount of space in the glomerular tuft not occupied by capillaries also seems quite variable in different animals. The recent careful investigation of the surface of a human

[4] In the reptiles and birds the urine coming from the ureters is fluid, and the final reabsorption of water takes place in the cloaca. Quite different figures have been given for the ureteral urine flow of the bird, none of which has been determined under strictly normal conditions. Gibbs (1928, 1929*b*) finds that extremely concentrated urine may come from the fowl's ureters. The fact that the ureteral urine is always extremely concentrated as regards waste products when compared to the urine of fresh-water fish and Amphibia, may be taken as supporting our hypothesis.

glomerulus by Vimtrup shows how erroneous are the estimates of glomerular surface used by Putter (1926) and von Mollendorff (1922).

Even with the number of glomeruli in a kidney determined, there is no exact method of correlating the number in different animals of widely varying size. No data exist to show on what basis such a comparison should be made. All one can do at present is to compare the number of glomeruli in the kidneys of animals of approximately the same size on the basis of body weight and then judge the relative glomerular surface by the average diameter of a glomerulus. An attempt at a more quantitative comparison of glomerular surface is in progress for fish, reptiles and birds of various sizes, and a further quantitative interpretation will be deferred until this work has been completed. For mammals (von Mollendorff, 1929) and Amphibia (Steinbach, 1927) the number and size of the glomeruli have already been determined for many species.

In conclusion, it may be remarked that the relative amounts of a substance eliminated by the glomerulus and tubule will depend on the glomerular development of the animal. If we accept the filtration theory of glomerular function, it is obvious that in eliminating water through the glomerulus, diffusible plasma constituents must also be eliminated and excreted unless reabsorbed by the tubule.[5] Looking at this problem from the standpoint of secretion by the tubule, it is clear that where a large amount of fluid is eliminated by the glomerulus, the secretory function of the tubule will be minimal, but where small amounts are eliminated in the case of poor glomerular development, secretion by the tubule will be maximum. This agrees fairly well with what we know about secretion by the tubule in different classes of vertebrates. Thus, in mammals, it has been supposed that filtration by the glomerulus and reabsorption by the tubule play a major rôle in the production of the urine, and that secretion exists only as a relic of a primitive process (Mayrs, 1924; Marshall, 1926). On the other hand, in birds there is now rather conclusive evidence (Mayrs, 1924; Gibbs, 1929*a*) that secretion plays a major rôle in the elimination of uric acid by the kidney. That secretion by the tubule also will play an important rôle in excretion by the reptilian kidney can be safely predicted. In Amphibia and fresh-water fish, where secretion by the tubule may be small, special conditions must be employed to demonstrate it. On the other hand, tubular elimination or secretion is easily proven in marine teleosts (Marshall, 1930). Just how much secretion by the tubule will take place in a kidney would appear, then, to depend on

[5] Of course, some reabsorption of water and concentration of urinary constituents takes place in the tubule of the lower vertebrates.

the amount of filtrate elaborated by the glomeruli and hence on glomerular development and activity.

Summary

Evidence is presented for the view that the glomerular development of the kidneys of vertebrates is related to water excretion. The protovertebrate kidney was at one stage probably aglomerular and the glomerulus was evolved as an adaptation to a fresh-water habitat. In the lower vertebrates remaining in fresh water (dipnoans, ganoids and fresh-water teleosts) and in those still in intimate dependence on it (Amphibia), the glomerular development is good; but with the secondary assumption of a marine habitat (marine teleosts) or with the assumption of terrestrial life in which water conservation becomes a necessity (arid-living reptiles and birds) the glomerular development is extremely poor. In the mammals (and possibly to some extent in lower vertebrates) the primitive water-excreting function of the glomerulus has been secondarily diverted to a filtration-reabsorption system designed to excrete waste products without the loss from the body of excessive quantities of water. The relative importance of tubular secretion in any kidney will, on this view, depend upon the extent of glomerular development.

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PLATE I

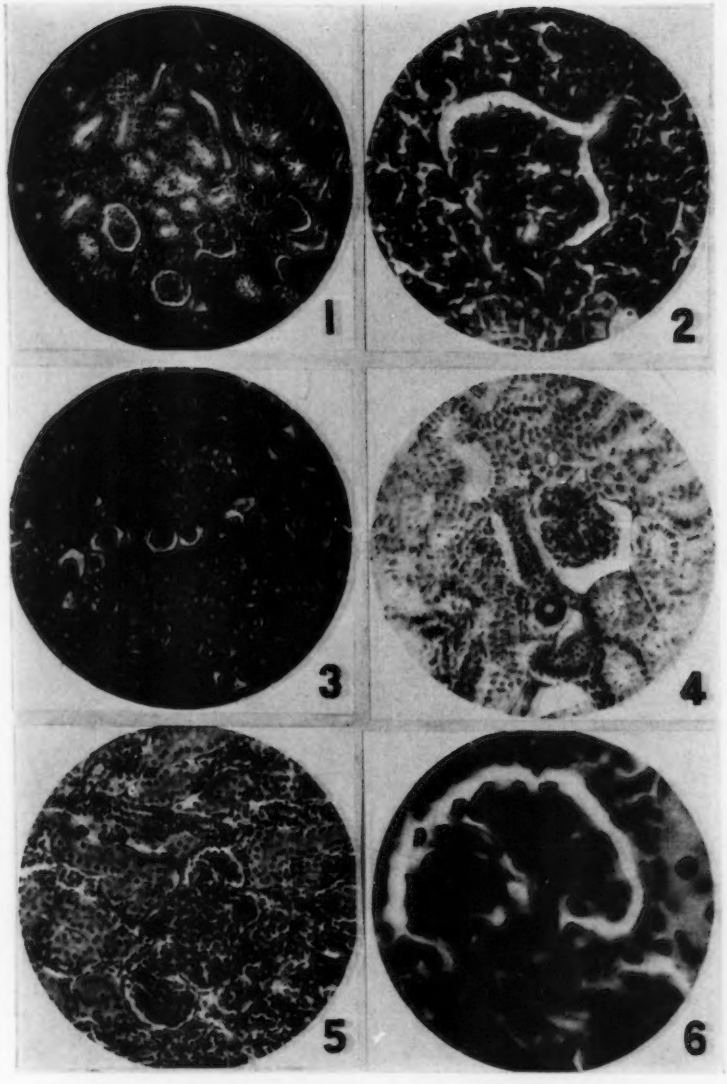

FIG. 1. Section of kidney of Cat fish (*Ameriurus nebulosus*), group I. × 63.
FIG. 2. Higher power of portion of field shown in Fig. 1. × 262.
FIG. 3. Section of kidney of Silver perch (*Morone americana*), group I. × 63.
FIG. 4. Higher power of portion of field shown in Fig. 3. × 262.
FIG. 5. Section of kidney of Lung-fish (*Protopterus aethiopicus*), group I. × 63.
FIG. 6. Higher power of portion of field shown in Fig. 5. × 262.

PLATE II

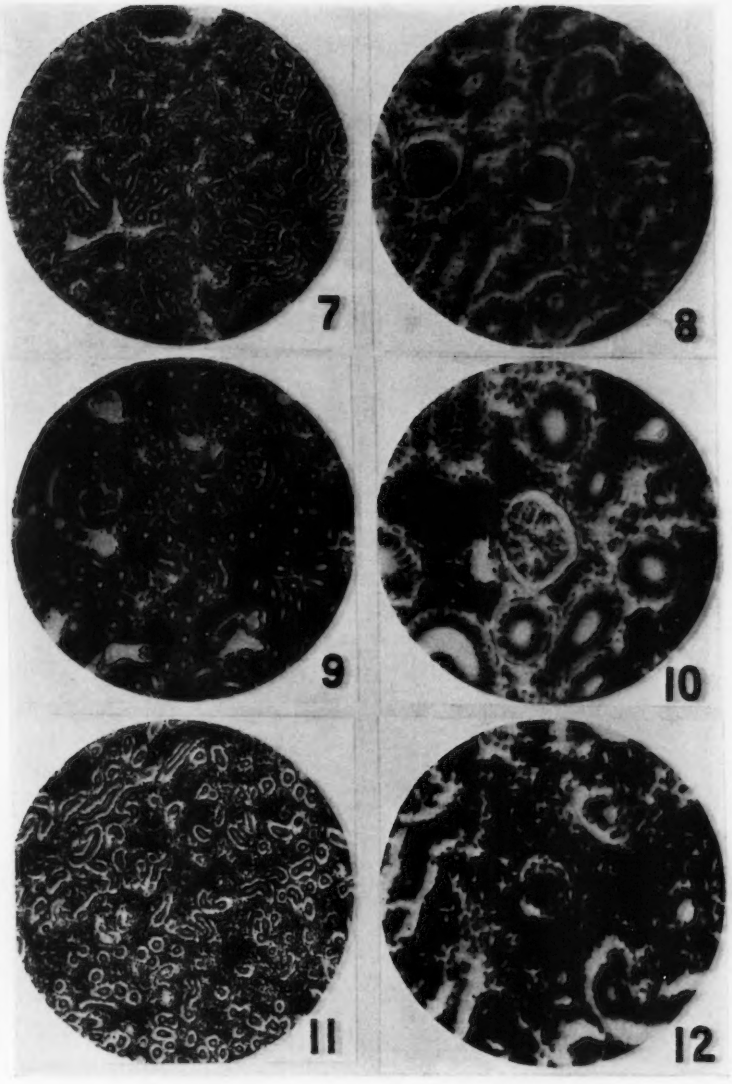

FIG. 7. Section of kidney of Jack (*Caranx ruber*), group II. × 63.
FIG. 8. Higher power of portion of field shown in Fig. 7. × 262.
FIG. 9. Section of kidney of Flounder (*Pseudopleuronectes americanus*), group II. × 63.
FIG. 10. Higher power of portion of field shown in Fig. 9. × 262.
FIG. 11. Section of kidney of Brown tang (*Teuthis hepatus*), group III. × 63.
FIG. 12. Higher power of portion of field shown in Fig. 11. × 262.

PLATE III

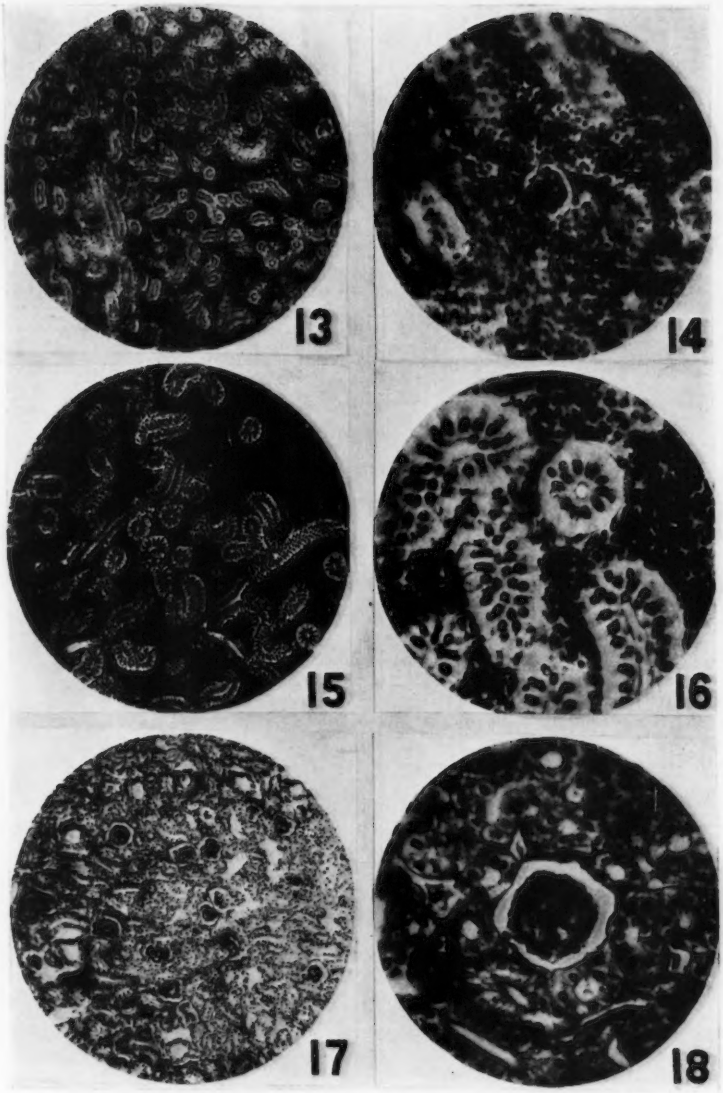

FIG. 13. Section of kidney of Shellfish (*Lactophrys bicaudalis*), group III. × 63.

FIG. 14. Higher power of portion of field shown in Fig. 13. × 262.

FIG. 15. Section of kidney of Toad fish (*Opsanus tau*), group IV. × 63.

FIG. 16. Higher power of portion of field shown in Fig. 15. × 262.

FIG. 17. Section of kidney of Terrapin (*Pseudemys sp.*). × 63.

FIG. 18. Higher power of portion of field shown in Fig. 17. × 262.

PLATE IV

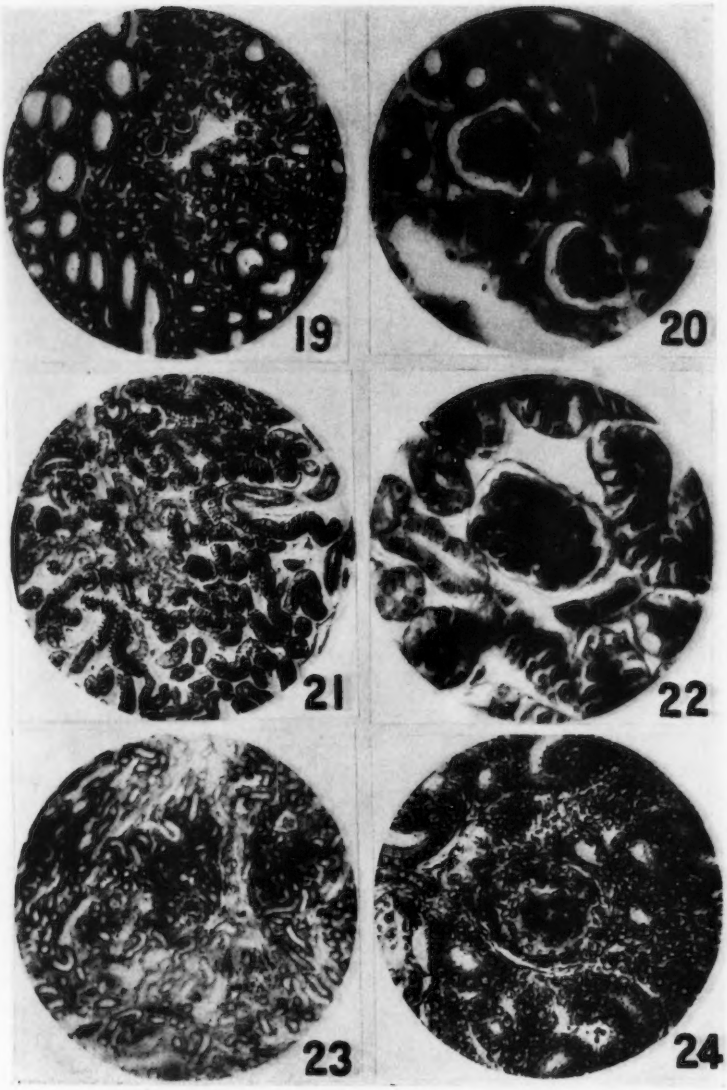

FIG. 19. Section of kidney of Horned toad (*Phrynosoma cornutum*). × 63.
FIG. 20. Higher power of portion of field shown in Fig. 19. × 262.
FIG. 21. Section of kidney of Boa constrictor (*Boa imperator*). × 63.
FIG. 22. Higher power of portion of field shown in Fig. 21. × 262.
FIG. 23. Section of kidney of chicken. (*Gallus domestica*). × 63.
FIG. 24. Higher power of portion of field shown in Fig. 23. × 262.

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THE FORMATION, REGENERATION, AND TRANSPLANTATION OF EYES IN PECTEN (*GIBBUS BOREALIS*)

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INTRODUCTION

One of the most highly organized and fascinating eyes among the invertebrates is found in *Pecten*, the common scallop. Especially good descriptions of the histology of the eye, which occurs along the mantle edge in the various species, may be found in the articles of Patten (1887), Hesse (1900), Hyde (1903), and Dahlgren and Kepner (1908). Drew (1906) also has a review of the structure in his excellent work on the giant scallop.

It was originally planned to find out if the mantle edge could be stimulated in any way to form eyes, or, in other words, to find the determining factor of these eyes. Before any information could be collected on the proposed experiment, various problems were confronted which necessitated a preliminary study. Among these problems were the following questions: (1) Do more eyes form as the individual grows and more space is provided for them? (2) How do the eyes develop? (3) Will the eyes regenerate? (4) Does the nervous system exert any qualitative or quantitative influence on the regeneration of the eyes? (5) Will the eyes grow and differentiate when transplanted to places other than the edge of the mantle?

These and innumerable other questions are of such interest that the results of the findings seem to warrant reporting.[1]

THE STRUCTURE AND THE FORMATION OF THE EYES

The eyes of *Pecten*, metallic in appearance, are scattered along the mantle edge. They are located on short stalks interior to numerous tentacles which are adjacent to the shell. The tentacles overhang the eyes in such a manner as to provide a shade for them. Sometimes the eyes are placed at almost equal distances from one another, but,

[1] The writer wishes to acknowledge, at this time, his indebtedness to Dr. H. B. Adelmann of Cornell University for suggesting this study.

for the most part, their arrangement is of an irregular nature. More eyes are always found on the left or upper mantle.

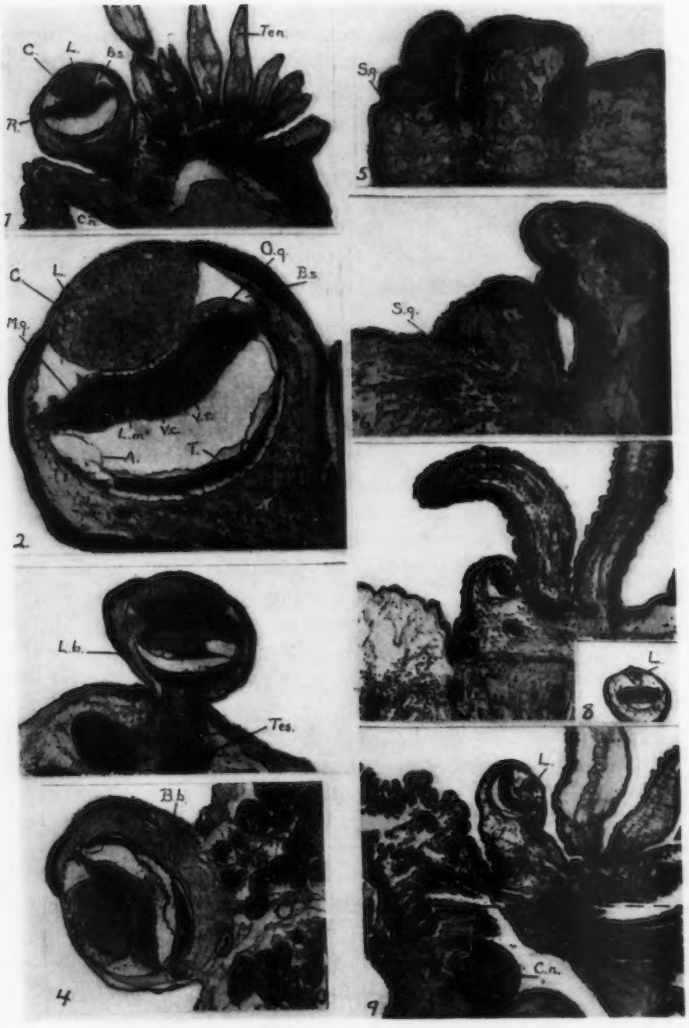
The eye of *P. gibbus borealis* consists of an epithelial covering which is modified into a cornea. Inside this covering we find a cellular lens, a blood sinus, the several layers of the retina, a space filled with vitreous humor, the argentea, and lastly the pigmented tapetum (Figs. 1 and 2).

The lens, oval in shape, is separated from the cornea by a thin layer of connective tissue. Its edges reach as far as the pigmented area of the cornea or iris. Its proximal curve, projecting into the blood space, almost touches the retina. Structurally, the lens consists of cells which contain peripherally located nuclei and a vacuolated cytoplasm.

The retina extends across the eye sac between the anterior blood space and the posterior vitreous humor. The thick layer of visual cells are found on the proximal surface of the retina. Their distal ends are in the form of long, thick, visual rods. The place where the visual cells are continued as the rods is straight and even and is marked by a set of fine plates in the substance between the cells. These plates constitute the limiting membrane (Fig. 2). Miss Hyde thinks that each rod in *Pecten irridans* has a nucleus and is separate from the visual cell. Dahlgren and Kepner did not find these nuclei in the rods of *Pecten tenuicostus*, and my preparations, although stained in iron hæmatoxylin, do not show separate components as described by Miss Hyde. Since the visual cells are on the proximal surface, the retina may be considered as inverted. In this respect, it resembles the vertebrate eye.

Lying distal to the visual cells of the retina is a layer of cells which constitute the outer ganglionic cell-layer. Dahlgren and Kepner find that nerve and supporting cells constitute this layer. According to them, neurofibrils enter it from the lateral branch of the optic nerve, pass through the cytoplasm of the nerve cells, and then ramify inwardly to send branching ends to the visual units. The visual cells also establish direct connection with large ganglionic cells along the margin of the retina (Fig. 2).

The optic nerve approaches the eye and divides into two branches, the basal and the lateral. The basal branch (Fig. 4) was thought by Miss Hyde to be connected with the marginal ganglionic cells, while the lateral division (Fig. 3) was related to the external ganglionic cell-layer. The basal branch was also considered by Miss Hyde to be afferent, while the efferent path coursed over the side branch to the external ganglionic cell-layer.


C.
L.
Bs
R.
Ten.
Ch.
1
Sq.
5
O.q
Mq.
L.m.
Vc.
V.c.
A.
T.
2
S.q.
6
L.b.
Tes.
8
L
Bb.
4
9
C.n.

One finds a variation in the number of eyes between individuals of the same size. More eyes are found in large than in small *Pecten*. The question at once arises whether or not new eyes form or whether they are always present yet in somewhat of a latent condition. Patten described briefly the embryology in 3 mm. *Pecten*. However, he did not investigate the problem just presented. Apparently, new eyes are not added in accordance with any particular plan, but make their appearance as space for them is provided.

The occurrence of eye formation in the middle of the shell is only occasional. Near the ears of the shell, one is more apt to find stages in development, showing that most of the growth must occur in this region. Stages of formation, of course, are more frequently found in smaller than in larger individuals.

At the inner edge of the tentacles one finds a shallow groove which may be called the ophthalmic groove (Figs. 5 and 6). It is at the base of this groove that formation begins. The first indication of an eye

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FIG. 1. Transection of the mantle edge to show the position and structure of a large eye. × 50.

FIG. 2. A magnified section to show the detailed structure of an eye. × 140.

FIG. 3. This photograph shows an eye which has developed in the gonad. It was transplanted at about the same stage as the one illustrated in Fig. 7. Note the lateral branch of the optic nerve which had grown about an inch into the gonad. × 80.

FIG. 4. This eye was transplanted to the gonad when about the size of the one illustrated in Fig. 1. Note that it has continued to enlarge and that the basal branch of the optic nerve has grown considerably. × 60.

FIG. 5. This photograph shows a stage in the development of an eye. The vesicle has differentiated from the downgrowth which occurred at the point *S.g.* × 95.

FIG. 6. A slightly later stage in the development of an eye. Note that the right side is thickening to form the retina, while the other side will thin to form the posterior part of the eye. × 95.

FIG. 7. This figure shows the retina and its development more distinctly. × 95.

FIG. 8. This section shows the beginning of the lens formation from connective tissue cells above the retina. × 95.

FIG. 9. Further differentiation of the eye is illustrated. Note also that the eye has been pushed outward as a result of the lengthening and rotation of its stalk. × 95.

The left side of Figs. 1, 2, 5, 6, 7, and 9 represent the inner side or toward the light while the right side of these figures is next to the shell or the outer side.

ABBREVIATIONS

A., argentea
B.b., basal branch of optic nerve
B.s., blood sinus
C., cornea
C.n., circumpallial nerve
L., lens
L.b., lateral branch of optic nerve
L.m., limiting membrane
M.g., marginal ganglionic cells
O.g., outer ganglionic cell-layer
R., retina
S.g., ophthalmic groove
T., tapetum
Tes., testis
V.c., visual cells
V.r., visual rods

is a downgrowth. These downgrowths may be hollow invaginations or nearly solid buds. The whole papilla then elongates, and gradually loses its connection with the surface epithelium (Fig. 5). Meanwhile, it has begun its transformation into an optic vesicle, the cells of which are distinctly provided with cell walls. Some cells are greatly pigmented, although pigmentation may be entirely lacking (Fig. 6).

An increase in the number of cells by mitosis soon occurs in the side of the vesicle which is farthest from the light (Fig. 7). The cells continue to increase in number on this side until several layers are present. Meanwhile, differentiation begins and the different layers of the retina appear. Those cells more central (with respect to vesicle) aline themselves as the visual cells, while those more peripheral form the ganglionic cell-layer (Figs. 8 and 9).

The single layer of cells, which forms the wall of the vesicle next to the light (Figs. 7 and 9), is first filled with colorless granules which gradually acquire a red color. These granules are located particularly in the periphery of the cell. Apparently the argentea is derived from the clear refractive distal or innermost part of these cells. Later it separates from the more pigmented peripheral portion, and becomes compressed into a thin layer which is devoid of pigment. The pigmented part persists as the definitive tapetum. Within the vesicle arises the vitreous network, a homogeneous fluid that has a vacuolated appearance.

As the retina continues to differentiate, it becomes concave (Fig. 9). Connective tissue intimately surrounds the optic cup. In the region of the concavity of the retina the connective tissue cells become pigmented and spherical. As the cells aggregate together, the nuclei become peripherally located, and gradually the lens appears (Figs. 8 and 9).

The eye begins its formation at the base of a groove, but with further development, it is pushed outward at the end of a stalk. The retina starts to develop turned away from the light, but as the stalk lengthens, it is rotated toward the light. With increase in size the eye occupies the entire end of the stalk.

The epithelium directly above the lens loses its pigmentation and forms the cornea. That at the edges of the lens, however, does the opposite, or becomes highly pigmented to form the iris. Nerves grow out from the ganglionic cells of the marginal and external ganglionic cell-layers in the direction of the circumpallial nerve.

Abnormalities in development frequently occur. Large pigmented undifferentiated vesicles are commonly encountered. Apparently the stimulus has been present and has caused the vesicle, but, for some

reason, there has been no further differentiation. One also finds eyes completely upside down, especially on short stalks near the ear of the shell. Externally, such an eye gives one the impression that the entire cornea is pigmented, but upon sectioning it one observes that it was the pigment layer of the eye which was outward. This is, no doubt, due to the fact that the stalk containing the eye did not lengthen sufficiently to allow for a rotation.

Duplication is, likewise, commonly encountered. Oftentimes a retina will have two concavities, each having a small lens. Two eyes, and even three are occasionally found on the same stalk. Observations show that such a condition resulted from the forking of the downgrowth in the beginning stage of development. The factor or factors which are influential in causing this condition have not been found.

Many irregularities are manifested in the rate of development. The optic cup may be nicely differentiated and the lens may be entirely lacking. The opposite extreme, or the early formation of the lens in the presence of small undifferentiated retina, also occurs, although not frequently.

A thorough search has given no insight on the stimulating factor in the formation of an eye. Pigmentation, nerve endings, attachment of muscles have all been carefully considered, yet these fail to give satisfactory reasons for the presence of the eye. The question regarding the determination of the eye will be considered more fully when discussing the regeneration of the eye.

The potentiality for forming eyes appears to be gradually lost as the *Pecten* become larger. The number in large individuals seems to be fairly constant.

The Influence of the Nervous System on the Regeneration

Before going further, it might be appropriate to outline briefly the plan of the nervous system of *Pecten*. At the sides of the gullet there are two distinct and separate cerebral ganglia which are united by a cerebral commissure. Each ganglion gives off a cord, the cerebro-pedal connective, which passes downwards and backwards to the pedal ganglion. From each cerebral ganglion there also proceeds directly backwards a long cerebro-visceral connective, which ends in a visceral ganglion placed on the ventral side of the adductor muscle. The visceral ganglia of the two sides are fused to each other by a ganglionic commissure. In the edge of the mantle or pallium, we find a nerve, the circumpallial nerve (Figs. 1 and 9), which contains some nerve cells. This nerve is connected with the visceral ganglion by a number

of nerves, the posterior pallial nerves, while anteriorly it receives a supply from the cerebral ganglion, the anterior pallial nerves.

Pertaining to the problem of regeneration, we are confronted with the following questions: (1) Will the eyes reappear upon removal? (2) Does the nervous system exert any influence on this reappearance? (3) What factors are responsible for the reappearance of the eyes?

One has to refer only to the literature to learn of the amazing number of articles pertinent to the subject of the influence of the nervous system on regeneration. Herbst (1916) found that the eyes of certain Crustacea regenerate normally only when the optic ganglion of the animal in question is not injured. When the optic ganglion was removed together with the eyes, heteromorphic bodies, having the strucure of feelers, were produced instead of eyes.

In Mollusca, the studies of König (1915) and Nonne (1925) on the gastropods is interesting. König found that in *Arion empiricorum* the regeneration of the eyes took place independent of the tentacle ganglion, cerebral ganglion, and nerves. More recently, Nonne observed that the eye of *Tachea hortensis* would regenerate if the corresponding cerebral ganglion were absent. He found that it took longer for the eye to regenerate on the injured side than it did on the normal side. Likewise, the eye often remained smaller. He concluded, therefore, that the nervous system had a quantitative influence on the eye regeneration rather than a qualitative. It was thought that it might be interesting to see if similar conditions existed in the other Mollusca, particularly the lamellibranchs (Pelecypoda) with which this investigation is concerned.

Normally, *Pecten gibbus* rests on its right side, which is usually somewhat lighter and less pigmented. One will find along the edge of the right pallium of a 3.5 cm. *Pecten* an average of about thirty-five eyes, while on the left or upper mantle forty-five is about the average number.[2] This size (3.5 cm.) usually has as many eyes as the larger *Pecten*, and, since they were more easily handled than larger ones, they were more frequently used for experimentation. However, much smaller and much longer *Pecten* were employed.

If one removes the eyes from one of the mantles of a 3.5 cm. *Pecten*, a similar number will reappear in about forty days. In smaller *Pecten* they reappear sooner, and in larger *Pecten* they reappear more slowly. Both sides regenerate in a similar manner when the eyes are removed from both mantles. These experiments establish rather definitely the fact that the eyes would reappear upon removal. Sections of the

[2] This measurement was recorded at the hinge.

mantle showed that the regeneration took the form of ordinary development which has previously been described.

Several possibilities existed wherein one might learn whether or not the nervous system exerted any influence on the reappearance of the eyes. One might remove part of the visceral ganglion that supplies pallial nerves to the circumpallial nerve. The pallial nerves and circumpallial nerve could also be cut.

In the cases where the visceral ganglion was removed from one side and the eyes from both sides, the eyes reappeared on both sides equally as fast. When compared to cases where only the eyes were removed, no difference was found in the time interval that it took for the eyes to form again. Removal of the eyes from both sides and the cutting of the pallial nerves from one side had no effect on the reappearance, for a similar number of eyes was found on both mantles after twenty days. Even cutting the pallial and circumpallial nerve on one side and removing the eyes from both mantles had no effect on the rate of appearance between the two sides. It was, likewise, found that it made no difference in the time necessary for reappearance if the eye was removed or the entire eye and stalk. As usual, a new eye formed by a downpocketing at the base of the groove.

These experiments might be subject to criticism since the circumpallial nerve contains ganglionic cells within itself and this had not been removed. In order not to leave this questionable, the eyes were removed from the entire mantle and about five-tenths cm. of the length of the mantle, excluding the ophthalmic groove, was cut away. The dotted line in Fig. 9 illustrates approximately the section that was taken away. The material removed was later sectioned to confirm that the circumpallial nerve had been extracted. Thirty-four days later the *Pecten* were examined and many eyes had reappeared on the mantle where the nerve had not been removed. Where the nerve had been cut away, the eyes were very small and in the first stages of development. They were also less numerous. Since the eyes were less numerous and less developed, the conclusion seemed justified that the nervous system did exert a slight quantitative influence on the reappearance. Disturbance of vascular relations could, however, have accounted for a portion of this extra time that it took for the eyes to reappear.

From all of the various experiments, one could, at least, conclude that the nervous system had little influence on the regeneration and development of the eyes and that the eyes were self-differentiating.

Transplantation of Eyes

In order to test the self-differentiating property of the eyes further, it was decided to transplant anlagen and fully developed eyes to places remote from their original location. The edge of the gonad was found to be a very desirable place to anchor and to grow the eyes.

The earliest identifiable stages (Fig. 7) were transferred to the edge of the gonad and, after an interval of three or four weeks, the gonads were removed and the eyes were found to have developed normally in every respect (Fig. 3). The nerve, both lateral and basal branches, had made a considerable growth into the gonad. Progressive and normal differentiation had occurred in all parts of the eye.

Large eyes, when transplanted to the edge of the gonad, retain their normal condition and in no way show signs of regression. Instead the eyes enlarged (Fig. 4) and the optic nerve made a considerable growth into the gonad.

When the optic cup was planted without the lens, the invading connective tissue cells were stimulated to take characteristics of lens cells. Portions of the optic cup and of the lens, however, degenerate when planted alone.

Discussion

It has previously been pointed out that Hyde thought that the retina of the eye was provided with two different branches of the optic nerve. The basal branch has been designated as afferent, while the efferent path coursed over the side branch. If the lateral branch were mainly efferent in nature, then one might think that the nerves constituting it would grow towards the eye. In the study of the embryology of the eye, I do not find that this occurs, but that the lateral branch grows away from the eye. This would indicate that the cell bodies of these fibres were in the retina. It appears that the external ganglionic layer of cells serves this purpose.

Further evidence that the cell bodies of the nerve fibres of the lateral branch are located in the retina is seen in transplanting the eyes. If these are entirely efferent fibres in the lateral branch, then one might expect them to degenerate or not develop when the eye is transplanted. This is not the case, however, as may be seen in Fig. 3. This photograph shows the lateral branch quite normal and making a growth into the gonad. Therefore, the lateral nerve ganglonic cells must be located in the retina. The fact that the ganglionic cells of the basal and lateral branches are located in the retina, does not give any insight as to their function. From observations, I believe that the lateral branch receives stimuli from the central part of the retina, while the

basal branch gets its supply from the margin of the retina. Dahlgren and Kepner apparently think that there are two different pathways for the impulse.

As to the function of the eyes, we know little. Patten found that *Arca noae* was extremely sensitive to light. Shadows and small objects, within two or three inches, caused it to close at once. It has been suggested that the eyes of *Pecten* may help them to avoid enemies. They may also prevent them from being left on dry land in case of tide. The growing intensity of light, in this case, would seem to act as a stimulating agent.

Why we have more eyes on the left or upper mantle is interesting. Is it because more eyes are needed to see moving objects on the bottom, or is it because there is less light and more eyes are needed for acuity? Until something is learned about the determining factor of the eyes, it will be difficult to give a hypothesis for the occurrence of more eyes on the upper side of the mantle. Similarly, it is difficult to arrive at any definite conclusion for the orientation of the eye in development. Why does it always develop away from the light?

Equally of interest and inexplainable is the presence of large undifferentiated vesicles. In this case the stimulus was apparently present at first and then subsided, or did the tissues have eye potentiality and then lose it?

As to the nature of the determining factor of the eyes, I am unable to state at present. Pigmentation, nerve endings, attachment of muscles, light, and injury are all suggested.

The epithelium covering the tentacles contains a variable amount of pigment. It was thought that the amount of pigment might in some way stimulate development. However, the downgrowths had about an average amount of pigment, and as far as I was able to determine, eye development occurred whether or not there was an abundance of pigment or a lack of it.

There was no evidence that any nerve had come in contact with the epithelium and had acted as a stimulating factor in producing a downgrowth. Even the eye vesicle had formed previous to the insertion of the retractor muscle into the edges of the cornea, and the muscles, or their attachment, therefore, seemed to have no part in the formation of the eye.

Whether or not light may in some way stimulate the epithelium is still to be investigated. It would appear that it did not, since the eyes develop away from the light.

It would seem that the only possible solution left was that the epithelium alone possesses the potentiality for forming eyes. This

power must be evenly distributed, since eyes appear almost anywhere. It would be interesting to transplant ophthalmic groove to gonad, to invert mantle edge, to transplant other epithelia to edge of the mantle in order to learn something about the determining factor of the anlagen of the eyes.

Even if this property of the epithelium for forming eyes is rather distributed, there must be some stimulus that evokes eye development.

Conclusions

1. Eyes continually form in *Pecten* as the individual grows and more space is provided for them.

2. Eyes will reappear upon removal, and the nervous system exerts very little influence on the reappearance.

3. Eyes and eye anlagen can be transplanted to other parts of the body. Transplanted eyes grow and retain a normal condition, and the optic nerve attempts to establish normal relations.

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REVERSAL OF PHOTOTROPISM IN A PARASITIC WATER MITE

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Reversal in the phototropic response of certain animals sometimes occurs in the course of normal development and has been brought about in the laboratory by mechanical stimulation, change in temperature, change in light intensity, starvation, and chemical stimulation. In many cases the reversals produced artificially are apparently non-adaptive, or at least of no evident use to the organism. While working with certain parasitic water mites it was found that material from the host causes a reversal in the light response which is evidently of an adaptive nature. The reversal was studied in normal animals in directional light and also in animals blinded in one eye. The destruction of one eye brings about circus movements which are reversed by an extract of materials from the host.

The mite used in the study was *Unionicola ypsilophorus* var. *haldemani* (Piers). It lives between the inner and outer gills of the common fresh water clam or mussel, *Anodonta cataracta* Say, and is commonly called a parasite although it may be only commensal with the clam. The number of mites found in an individual clam varied from one to fifteen, the average for a large number of clams being 5.3 mites. Only very rarely were clams found without some mites present. According to Wolcott (1898) these mites seldom leave the host. However, they must do so at times, otherwise young clams would not become infested. During the course of the experiments specimens of *Anodonta* were kept under various conditions of light, temperature, and oxygen tension but on only two occasions were mites known to leave the clams. They seem to feed largely on the mucus from the gills and their presence in large numbers appears to increase the amount of mucus secreted.

When removed from the gills and allowed to remain in water from the mantle cavity, the mites are indifferent to directional light, wander aimlessly about the dish, and tend to cling to bits of mucus and gill. If washed and placed in fresh water free of material from the host they show a definite positive phototropism. Among a large number of individuals a few may remain indifferent to light but the majority

show a strong photopositive reaction an hour or so after removal from the host.

When a number of mites are placed in an aquarium with a clam they soon find their way inside the clam. In one case forty-five mites were placed in a small aquarium with a single clam. The following day only two could be found free in the aquarium. The clam was opened and forty-eight mites were removed from between the gills, all but five of these having gained entrance to the clam over night.

The problem presented by the behaviour of the mites was to explain why they remain within the host although photopositive after removal, and how they are attracted to the host when free in an aquarium.

When a number of mites are placed in water containing pieces of gill they soon locate the gill, particularly if in the dark or in diffuse light. They tend to crawl into the gill by way of the cut edges or to collect under the pieces, there remaining quiet. This would seem to indicate a positive stereotropism. It is believed that a definite chemical stimulus directs the mites to the material from the host and that this attraction aided by a positive stereotropism keeps the mites within the clam even when a strong beam of light is projected into the mantle cavity.

During the early observations on the behaviour of these mites another interesting phenomenon was noted. Mites which were distinctly positive to light exhibited an immediate reversal to a photonegative state when transferred to a water extract of clam gill or to water from the mantle cavity of the clam. This reversal was at times only temporary but might last for a period of an hour or more. It was more pronounced and lasted longer if the mites had been out of the host for two or three days.

An experiment to show the effect of dilution of gill extract on the reversal of the light response illustrates the usual type of reaction.

Four pairs of gills were ground in a mortar with 20 cc. of distilled water. The mixture was centrifuged, the liquid decanted and filtered. 5 cc. of this extract, which for convenience may be called a 100 per cent solution, were placed in a small rectangular glass jar, 8 cm. long and 2 cm. wide. This was illuminated from one end with a beam of light from a 25 watt lamp at a distance of 15 cm., projected through an opening 2 cm. in diameter in an opaque screen. The light was reduced in intensity by interposing a sheet of tracing paper between the lamp and the jar. The end of the jar away from the light was covered on the inside with dull black paper coated with paraffin in order to prevent reflection of light. Ten photopositive mites were placed in the end of the jar nearest the source of illumination and their reactions noted.

For each dilution of extract a fresh lot of mites was used in order to avoid an accumulative effect.

Following are the results of using varying strengths of the original extract from 100 to 0.01 per cent:

100	per cent	10 mites immediately negative
50	per cent	9 mites immediately negative
25	per cent	8 mites immediately negative
10	per cent	8 mites immediately negative
5	per cent	5 mites immediately negative
2.5	per cent	6 mites negative (an obvious increase in the reaction time before the mites begin to move away from the light).
1.0	per cent	4 mites negative
0.05	per cent	2 mites negative
0.025	per cent	2 mites negative
0.01	per cent	0 mites negative

Only those mites which moved away from the lighted end of the jar and remained negative to light for an appreciable length of time were considered to have undergone a reversal in their response to light.

In control experiments handling and mechanical disturbance as well as the addition of small amounts of CO_2 were found to have no effect on the light response.

The reversal of circus movements of the mites confirmed the results obtained with directional illumination and offers a more striking demonstration of a change from a photopositive to a photonegative condition in an organism. Mites were blinded in the right eye by touching it with the point of a hot needle. The constant movement in circles toward the left or normal eye was sufficient indication that the blinding of the right eye had been successful. These animals showed remarkably uniform movements, travelling slowly, on ground glass, in circles with a diameter of 0.5 cm. When an extract of gill was added to the water near the mites, or when they were transferred to an extract of gill, an immediate reversal in the direction of circling would usually take place and the movement would be toward the blinded eye, indicating a change from a positive to a negative condition. Occasionally there would be failure to reverse or instead of complete reversal the movements would be at random.

An attempt was made to determine the nature of the substance in the gills which brings about the reversal in the light response and also exerts an attraction on the mites. Putrefaction at a temperature of 37.5° C. for a week was found to have no effect. At the end of this time the extract was as effective as fresh extract in causing a reversal.

Boiling for five to ten minutes likewise had little or no effect on the material which acts upon the mites. It is possible that decomposition products of the mucus or other proteins of the gills, which would not be affected by boiling or putrefying, are responsible for the reversal of the light response.

The organs concerned with the reception of the stimuli were likewise not determined. The removal of the labial palps at their basal joints in a number of mites had no apparent effect on the reversal or in the ability of these mites to locate and enter clams when free in an aquarium. As little is known concerning the chemical receptors in spiders and mites, it is difficult to say what centers are involved in the reversal.

Discussion

The behaviour of the variety of *Unionicola* which was studied seems to indicate a change from a primitive photopositive to a photonegative condition, due to a parasitic or commensal life. Negative phototropism assisted by chemotropism and stereotropism keeps the mites within the host. Upon removal from the influence of the clam, or material extracted from the clam, the mites revert to a photopositive state.

The rapid reversal from a photopositive to a photonegative condition when stimulated by extract of gill, or by water from the mantle cavity, is probably a central nervous phenomenon, and might be called a "conditioned reflex." Doubtless many parasitic animals become conditioned to stimuli from the host and show adaptive reactions quite different from their primitive responses.

Arey and Crozier (1921) investigated a case of change from independence of heliotropism to negative phototropism in *Onchidium,* a pulmonate mollusc, and their results are summed up in the following statement: "When tested apart from their specific normal environment the *Onchidia* are always negatively phototropic. In the natural state their movements are entirely independent of heliotropism . . . the simultaneous return to the nest on the part of the various members of a colony can be understood on the assumption of a 'reversal of inhibition' brought about by substances derived from materials while feeding." This case, while not identical with that described above, does indicate an effect of some substance in the food or in the environment on the light response of an animal.

Cole (1922–23) investigated the light response of *Limulus* and found that it might be modified by (*a*) fright, (*b*) hunger, (*c*) stereotropism, (*d*) photokinesis, and (*e*) unknown stimuli. He states: "It becomes clear after observing *Limuli,* however, that they are fundamentally and primitively positive to light, but that many factors may modify or mask the phototropic reaction."

Stier (1926) studied a case of reversal of phototropism in *Diemyctylus*, the spotted newt, and found that negative phototropism is characteristic of animals from which food has been withheld, while feeding reverses the response.

Summary

1. The water mite *Unionicola ypsilophorus var. haldemani* (Piers), living between the gills of the fresh water clam *Anodonta cataracta* Say, shows a positive reaction to light after removal from the clam.

2. An extract of the gills, or water from the mantle cavity of a clam, will cause a reversal to a photonegative state.

3. This reversal may be considered adaptive, for, aided by a positive chemotropism and stereotropism, it enables the mites to enter and remain within the host.

4. The phenomenon due to the parasitic life may be described as a "conditioned reflex"; the positive response to light being primitive and the negative response more recently acquired.

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REFLEX CARDIAC AND RESPIRATORY INHIBITION IN THE ELASMOBRANCH, *SCYLLIUM CANICULA*

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In the higher vertebrates the effect of stimulation of sensory nerves on the medullary centers is rather complex since either acceleration or inhibition of the heart may be obtained, often associated with acceleration or inhibition of respiration. In mammals Brodie and Russell (1900) found that stimulation of the pulmonary afferent fibers is more effective in causing reflex inhibition of the heart than excitation of afferent cardiac fibers, and the visceral supply is least effective. Bainbridge (1920), working also on mammals, obtained no reflex cardio-inhibition from the afferent pulmonary fibers, but did get marked respiratory inhibition. He found no evidence that the cardio-inhibitory center and the respiratory center are associated except through blood pressure changes. Alterations in blood pressure in the medulla have sometimes obscured true reflex processes. Thus Anrep and Starling (1925) offered evidence to show that inhibition of the heart in mammals could not be obtained reflexly through afferent fibers from the heart and aorta, and pointed out that an increase in blood pressure in the medulla caused cardiac slowing. These results contradicted in part the conclusions of Eyster and Hooker (1908), who believed that not only increased blood pressure in the medulla caused cardio-inhibition, but that reflex slowing of the heart could arise from the aorta, with both afferent and efferent pathways in the vagus. In 1926, however, Anrep and Segall confirmed the latter authors. Heymans (1929) has found cardio-inhibition in dogs to arise reflexly from increased blood pressure in the carotid sinus.

A survey of the literature on fishes, both cartilaginous and bony, gives one the impression that there is a nervous association between the respiratory rate and the heart rate. Schoenlein and Willem (1894) said that cessation of respiration in *Scyllium canicula* and *Torpedo ocellata* caused a reflex from the pharyngeal cavity which inhibited the heart. Bethe (1903) and Baglioni (1907) have found respiratory and cardiac inhibition occurring together in selachians. Lyon, (1926) studying blood pressure and respiration in sand sharks (*Carcharias*), noted that a great variety of stimuli applied externally or to certain

viscera caused inhibition of the heart and some sort of respiratory response. He pointed out an intimate relationship between the respiratory rate and the heart rate through the vagus.

Since neither vasomotor nerves nor accelerator fibers have been demonstrated in elasmobranchs (Schoenlein, 1895; Bottazzi, 1902; Müller and Liljestrand, 1918; Lutz, 1930*a*), one might expect to find in these fishes an association between cardiac and respiratory inhibition without the complicating blood pressure factors found in mammals. In elasmobranchs it is also possible, because of the persistence of the functions of the medullary centers without a blood supply, to investigate the influence of stimulation of sensory fibers from the heart without involving blood pressure changes.

Material and Method

Vigorous specimens of the dogfish, *Scyllium canicula,* averaging 300 grams, taken during the months of March and April from the Bay of Naples, were used. One specimen of *Scyllium catulus* (625 grams) was put through the usual procedures carried out on *S. canicula* and was found to respond in a similar fashion, but the smaller species was more easily handled and more abundant. The fish was removed from the water and the forebrain was separated from the rest of the brain by a transverse cut just in front of the optic lobes and destroyed. The spinal cord was transected at a desired level and pithed posteriorly. Transection at a high level, namely, between the fifth and sixth vertebrae or even more anterior, was found desirable, when the details of the experiment permitted, in order to keep the fish quiet. It was then placed ventral side up on a fish holder and the gills were perfused through the mouth. Respiratory movements soon became regular. The heart was exposed through the ventral wall of the pericardial chamber and a small gold hook was put through the superficial tissue at the tip of the ventricle. By means of a thread and a light writing lever its movements were recorded. A similar hook passed through the anterior border of the third or fourth gill slit was attached to a thread, passed horizontally to a pulley, and up to a light writing lever for recording respiration.

When necessary the vagus and its branches, including the beginning of the lateral line nerve and the last four branchial nerves, and the hypobranchial nerve were exposed quickly by cutting a slit with the scissors through the skin beginning above the fifth gill slit about 3 mm. ventral to the lateral line and running anteriorly toward a point just above the posterior angle of the eye. This opened into the fascia between the dorsal musculature and the gills and directly over the anterior

cardinal sinus, which was slit lengthwise exposing the nerves on its floor. This involved some loss of blood, and care was taken to keep the fish in a horizontal position to prevent sucking air into the heart. A bloodless method, described by Hemmeter (1912), of exposing "the ramus cardiacus" was found not to be practicable on the 300 gram fish, especially since the heart of *Scyllium canicula* receives not only a branch from the visceral trunk of the vagus but also a branch from the fifth branchial nerve (4th branchial division of the vagus).

The first large sympathetic ganglion was exposed when necessary by opening the abdominal cavity. Since this ganglion lies within the posterior cardinal sinus, the latter must be opened with considerable unavoidable loss of blood.

Results

Scyllium canicula was found to be very resistant to experimental procedures out of water when the gills were perfused. A preparation with the brain destroyed anterior to the optic lobes, the cord pithed posterior to the third vertebra, and the pericardial chamber opened, gave good cardiac and respiratory reflexes for over twenty-four hours. The laboratory temperature was about 14° C. When the anterior cardinal sinuses were opened, involving considerable bleeding, the

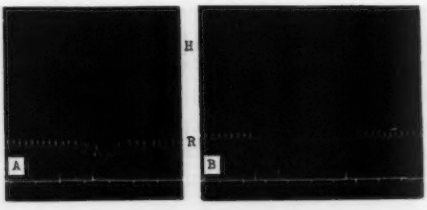

Fig. 1. Reflex cardiac and respiratory inhibition after transection of the ventral aorta. Forebrain and cord from the fifth vertebra destroyed. In this and subsequent figures the uppermost tracing is the heart record (*H*), the middle is respiration (*R*), and the lowest is the signal and time record. The large divisions are ten seconds. *A*, right fin pinched 21 minutes after transection of the aorta. *B*, gill perfusion stopped and started 31 minutes after transection.

preparation remained experimentally useful for about two hours. If in addition to the above procedures the abdominal cavity was entered and the posterior cardinal sinuses opened, medullary reflexes could be obtained for over an hour. In the latter case bleeding was profuse. When the aorta was transected, the respiratory center and the cardio-inhibitory center remained functional for over one hour (Fig. 1). Constant perfusion of the gills was found necessary to insure a quiet preparation, but without perfusion and with an intact circulation the

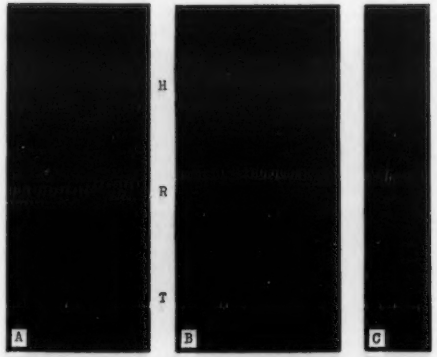

FIG. 2

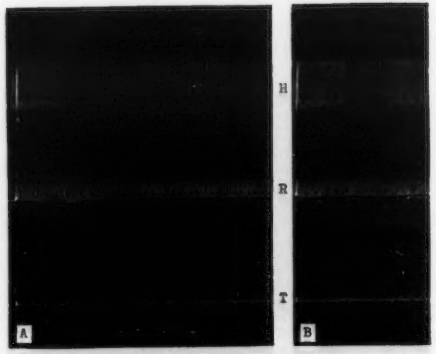

FIG. 3

FIG. 2. Cord pithed from the fifth vertebra. *A*, snout pinched gently with the fingers. Note reflex cardiac inhibition without respiratory inhibition. *B*, snout pinched vigorously. *C*, second right gill slit spread with forceps.

FIG. 3. Reflex cardiac inhibition without respiratory response. Cord pithed from the fifth vertebra. *A*, sides pinched with forceps dorsal to the pelvic fins. *B*, skin pinched on mid-abdomen.

medullary centers showed inhibitory reflexes for over three and one-half hours.

Cardiac and respiratory arrest or slowing were obtained with the cord pithed posteriorly from the second vertebra on mechanical and

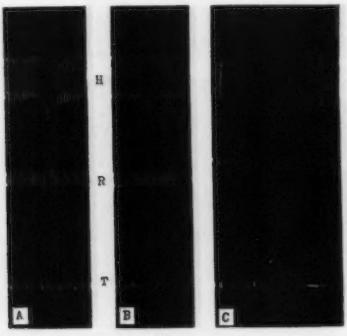

FIG. 4. Cord pithed from the fifth vertebra. *A*, needle stimulus to the left wall of the pericardial chamber. *B*, needle stimulus to the posterior wall. *C*, faradic stimulation of the posterior wall.

faradic stimulation of the skin of the head, nasal organs, gills, pectoral fins, cut surface of the coraco-mandibular muscle, pericardial walls, surface of the ventricle, oesophagus, stomach, spiral valve, and mesentery (Figs. 2, 3, 4 and 5). With the entire cord intact, similar

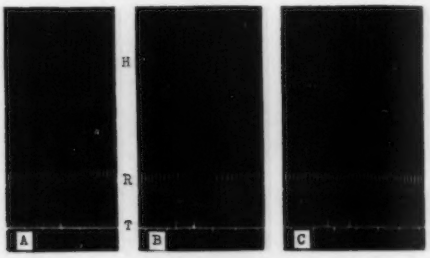

FIG. 5 Cord pithed from the third vertebra. *A*, stomach handled. *B*, posterior end of the stomach pinched with forceps. *C*, spiral valve pinched with forceps.

responses could be obtained from the kidney, epididymis, ovary, and uterus. No response could be elicited from the liver or testis even with the entire cord intact.

Faradic stimulation of the central end of the cut vagus, either the cardiac branch of the visceral ramus, the visceral ramus itself, or the cardiac branch of the fourth branchial division, the central end of the cut hypobranchial nerve, and the lateral line nerve (Fig. 6) produced cardiac and respiratory inhibition.

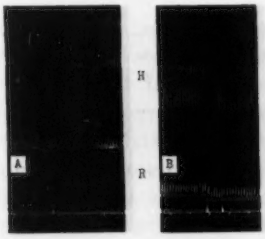

FIG. 6. Cord pithed from the eleventh vertebra. *A*, faradic stimulation of the central end of the left lateral line nerve. *B*, stimulus reduced.

Reflex cardiac inhibition and a reflex respiratory response were obtained when the rate of flow of the water to the gills was suddenly altered. The immediate response to starting and stopping the flow or to increasing and decreasing suddenly the rate was respiratory inhibi-

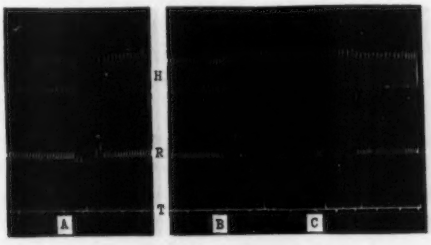

FIG. 7. Cord pithed from the fifth vertebra. *A*, water to the gills turned suddenly off and on. *B*, rate of flow of water increased and decreased to original rate. *C*, water gently stopped and started. Note respiratory inhibition without cardiac response.

tion, usually followed by one or more rapid and vigorous contractions of the gill muscles. The heart generally, but not always, showed inhibition as well (Fig. 7). This response occurred also after the aorta had been cut to prevent any possible blood pressure effects through changes in water pressure on the gill vessels.

When the sensory stimulation was strong or prolonged, both cardiac and respiratory inhibition occurred. Each effect, however,

could be produced independently. If the stimulus was weak or brief, especially when applied to the skin, stomach, or spiral valve, only a cardiac response was elicited (Figs. 2*A*, 3, 4*B*, 5*C* and 6*B*). But a weak stimulus to the gill region frequently produced only respiratory inhibition. This occurred sometimes when the flow of perfusion water was altered (Fig. 7*C*).

Mechanical stimulation of the sides of the pericardial chamber by stroking with a sharp needle gave marked cardiac inhibition (Fig. 4). Weak faradic stimulation of the posterior wall of the chamber for a few seconds frequently gave an inhibitory effect lasting a half minute or more. On the other hand, pinching the sides of the body at the level of the pectoral fins vigorously with the forceps for twenty-five seconds stopped the heart for a short period, but it escaped from complete inhibition while still being reflexly stimulated.

When the surface of the ventricle was pinched lightly with small forceps, it immediately contracted and then became completely inhibited in diastole for 30 seconds accompanied by inhibition of respiration in relaxation and by movements of the head. If the ventricle was allowed to beat against a sharp needle, it contracted quickly a few times almost stopping in systole, then became completely inhibited in diastole, generally associated with respiratory inhibition. If the needle was inserted carefully under the epicardium, there was no systolic inhibition or respiratory response, but complete diastolic inhibition and slow recovery. Reflex diastolic inhibition from mechanical stimulation of the ventricle was also obtained after the aorta had been cut. A weak brief faradic stimulus applied to the surface of the ventricle caused immediate fibrillation under the electrode and, when the stimulus was removed, marked diastolic inhibition. Reflex cardio-inhibition from the ventricle was also obtained with only the post-branchial branches of the fourth branchial divisions intact. With the vagi cut or the heart excised, mechanical or faradic stimulation of either the auricle or the ventricle did not inhibit in diastole, but faradic stimulation along the Cuverian duct was immediately effective in producing this type of inhibition.

Discussion

While the results reported above indicate that respiratory and cardiac inhibition usually occur together, they show no evidence that there is a dependence of the one on the other. Each may occur independently. Moderate or strong stimulation, however, at any point mentioned above, except the liver and the testis, invariably caused both cardiac and respiratory inhibition. The liver and testis apparently have

no sensory supply, stimulation of which gives either cardiac or respiratory responses.

Although stimulation of the central end of the lateral line nerve cut either at the gill region or midway between the pectoral and pelvic fins, gave both inhibitory responses, neither MacWilliam (1885) nor Kolff (1908) obtained heart reflexes on stimulation of this nerve in teleosts. Parker (1909) noted a temporary cessation of respiration on applying pressure to the lateral line region of the elasmobranch *Mustelus canis.*

The cardio-inhibition obtained on mechanical or electrical stimulation of the surface of the ventricle was undoubtedly of reflex nature since it was often accompanied by respiratory inhibition and skeletal muscular activity, responses which in themselves indicate afferent fibers from the ventricle. None of these responses was obtained with all of the cardiac branches of the vagi cut. In a paper on the innervation of the heart of *Scyllium* (Lutz, 1930*a*), further evidence is presented which indicates that the afferent pathway is in the vagus.

That reflex cardiac and respiratory inhibition should be so easily evoked by sensory stimulation of almost every part of *Scyllium* is interesting in view of the well-developed chromaphil system found in elasmobranchs and the inhibitory effect of adrenaline on the perfused heart of *Scyllium* described by Macdonald (1925). A similar effect of adrenalin chloride on a sinus-auricle preparation of various species of skate (*Raia*) and the dogfish, *Squalus acanthias,* has been described by Lutz (1930*b*). In view of the poorly developed sympathetic system (Müller and Liljestrand, 1918) and the lack of vaso-constrictor nerves (Schoenlein and Willem, 1894, and Schoenlein, 1895) found in elasmobranchs, the writer suggests that these inhibitory responses may be the expression of an emergency function which serves the fish in case of injury, especially to the gill region, and he has discussed the idea elsewhere (Lutz, 1930*b*).

I wish to thank Dr. Reinhard Dohrn and Dr. Enrico Sereni for the many courtesies extended at the Zoölogical Station, Naples, and Edna B. Lutz for technical assistance.

Summary

1. Mechanical and electrical stimulation of various parts of the surface of the body and certain viscera resulted in cardiac and respiratory inhibition when the spinal cord was pithed posterior to the second vertebra in *Scyllium canicula.*

2. Faradic stimulation of the central end of the cut vagus, either

the cardiac branches or the visceral ramus, and the central ends of the cut hypobranchial and lateral line nerves produced reflex cardiac and respiratory inhibition.

3. Stopping or starting the perfusion water through the gills or suddenly altering its rate of flow resulted in respiratory inhibition and sometimes cardiac inhibition.

4. Mechanical or electrical stimulation of the surface of the ventricle produced reflex cardio-inhibition and sometimes respiratory inhibition.

5. When stimulation was strong or prolonged, both cardiac and respiratory inhibition occurred, but weak stimulation of the gills or pharynx frequently produced only respiratory inhibition, whereas weak stimulation of the skin or viscera often produced only cardiac inhibition.

6. Both cardiac and respiratory inhibition were obtained for over twenty-four hours when the gills were perfused; for over three and one-half hours without perfusion; and for over one hour with the aorta transected at the heart. These phenomena, therefore, are true reflexes and are not due to alterations in blood pressure in the medulla.

7. The emergency function of the inhibitory responses is pointed out.

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RESPIRATORY RHYTHM IN THE ELASMOBRANCH, *SCYLLIUM CANICULA*

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The ease with which respiratory reflexes may be elicited by stimulation of the surface of the pharynx and gills in fishes led early investigators to look for a reflex control of respiration in these vertebrates. Schoenlein (1895) perfused the gills of *Torpedo* and *Scyllium* through the spiracle and found the respiratory rate to increase with increased flow of water until, with too great a flow, the regular rhythm ceased and ejection reflexes occurred. If the flow of water was stopped, there were a few respiratory movements until all the water in the gills was pressed out, when complete passive inhibition of respiration occurred. Since this author observed no signs of dyspnea during perfusion with oxygen-free sea water for twenty minutes, he came to the conclusion that the control of respiration was mainly reflex. Bethe (1903) perfused the gills of *Scyllium canicula* and *S. catulus* both with oxygen-free sea water and with sea water saturated with carbon dioxide, and found that the rate and strength of respiration remained unaltered for about forty minutes, after which it began to be slower and weaker. He also paralyzed the peripheral receptors of the gills and pharynx with cocaine and obtained a cessation of respiration. He therefore concluded that the gas content of the blood, which in the higher vertebrates plays such an important part, is without influence in fishes, and that breathing is caused by peripheral stimulation.

Since these conclusions contradicted the results of Duncan and Hoppe-Seyler (1893), who determined the level to which the oxygen content of the water could fall before signs of disturbed respiration occurred in fishes, Baglioni (1907–08) investigated the reflex respiratory mechanism in both teleosts and elasmobranchs. He found that the respiratory inhibition which occurred on stopping the flow of water was not absolute, and he believed it to be a reflex due to air in the pharynx. He also found that fishes which were allowed to swim freely in evacuated sea water showed a marked increase in respiratory rate, extreme dyspnea, and periodicity. He concluded that it is a

general property of the central nervous system in all vertebrates to react to oxygen lack by increased irritability.

The present paper is concerned with the respiratory responses which occur when the flow of blood through the respiratory center is stopped, and also with the effect of altering or stopping the flow of water through the gills.

Method

Vigorous specimens of the dogfish, *Scyllium canicula*, averaging 300 grams, taken during the months of March and April from the Bay of Naples, were used. The fish was taken out of water and the fore-brain was separated from the rest of the brain by a transverse cut just in front of the optic lobes and destroyed. The spinal cord was transected at various levels between the third and twelfth vertebræ and pithed posteriorly in order to keep the fish quiet. The animal was then placed ventral side up on a fish holder and the gills were perfused through the mouth. Respiratory movements soon became regular. The heart was exposed through the ventral wall of the pericardial chamber and a small gold hook was put through the superficial tissue at the tip of the ventricle. By means of a thread and a light writing lever its movements were recorded. A similar hook passed through the anterior border of the third or fourth gill slit was attached to a thread, passed horizontally to a pulley, and up to a light writing lever for recording respiration.

The perfusion water was taken from three large glass bottles placed 60 cm. above the fish holder. The rate of flow was controlled by means of a glass cock on each outflow tube near the bottle, and another near the mouth of the fish.

In certain experiments the aorta was transected at its junction with the conus arteriosus.

Results

A sudden alteration in the rate of flow of water to the gills evoked a reflex respiratory response and generally cardiac inhibition. The immediate effect of suddenly stopping and starting the flow or of increasing and decreasing the rate was respiratory inhibition, usually followed by one or more rapid and vigorous contractions of the gill muscles during which water was ejected from the mouth as well as from the gill slits. This ejection reflex occurred both when additional water was suddenly turned on and when the water was suddenly stopped (Fig. 1, *A* and *B*). A similar response to abrupt alteration in water supply to the gills occurred after the aorta had been transected

to prevent any possible blood pressure effects through changes in water pressure on the gill vessels.

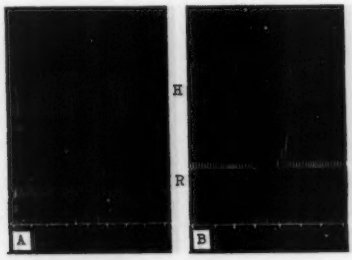

FIG. 1. Reflex cardiac and respiratory responses. In this and in subsequent figures the upper tracing is a record of the heart beat. The middle tracing records respiration. The large divisions on the time-signal record are ten seconds. *A*, perfusion of the gills suddenly stopped and started. *B*, flow of perfusion water quickly increased and decreased.

When the rate of flow was changed gently from 200 cc. per minute to 400 cc., there was an increase in both respiration rate and heart rate. If the flow was then reduced to the original, there was a return to the previous heart and respiratory rates (Fig. 2). In this case the heart rate before the change was 25 per minute, and the respiratory rate was 21. During a flow of 400 cc. per minute they were 42 and 27, respectively. A return to 200 cc. per minute gave 22 and 21, and a second increase to 400 cc. resulted in a heart rate of 42 and a respiratory rate of 31. Too great an increase, however, produced an irregularly inhibited rhythm of both the heart and the respiration.

When the flow of water was stopped for a period of several minutes, there was usually a temporary inhibition of both respiration and heart, followed by a slower rhythm which was sometimes very regular (Fig. 3) and sometimes showed a tendency toward periodicity (Fig. 4). With further continued lack of water the regularity of respiration was interrupted from time to time by generalized reflex muscular responses from the remaining parts of the central nervous system, during which both respiration and heart were irregularly inhibited. The medullary centers remained reflexly functional for over three and one-half hours.

The immediate result of transection of the aorta at the conus arteriosus was inhibition of both respiration and heart. But in a minute or two both began rhythmical activity although somewhat slower. Even without perfusion of the gills, regular respiratory movements occurred for a variable length of time. Figure 5*A* shows the

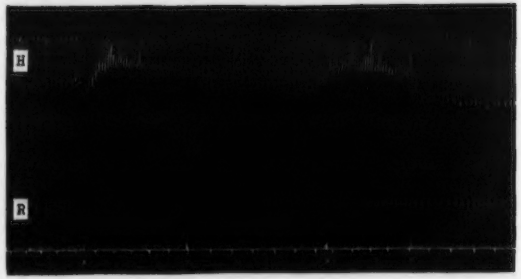

FIG. 2. Cardiac and respiratory responses to changes in the rate of flow of perfusion water to the gills. *X*, from 200 cc. per minute to 400 cc. *Y*, from 400 cc. to 200 cc.

FIG. 3. Perfusion water to the gills stopped from *X* until *Y*, a period of 6 minutes and 32 seconds. Regular respiratory rhythm.

regular respiratory rhythm becoming periodic about ten minutes after transection of the aorta. Figure 5*B* was taken from the same fish

FIG. 4. Perfusion water to the gills stopped at *X*. Onset of periodic respiration after 90 seconds.

fifty-three minutes after the aorta was cut. A periodic series of dyspneic respiratory movements occurred followed by a weaker and

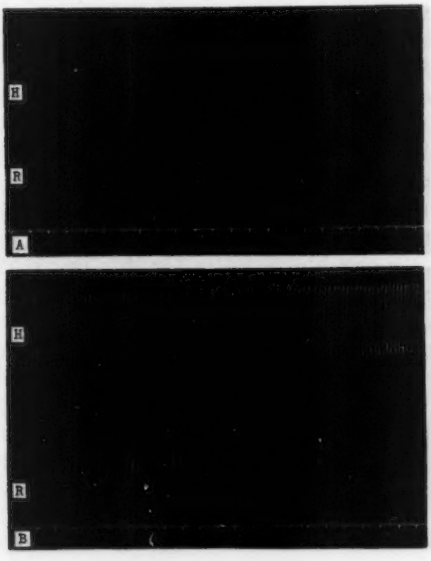

FIG. 5. Aorta transected at the conus arteriosus and perfusion of the gills stopped at the same time in a fish with the cord destroyed posteriorly from the fourth vertebra. *A*, record of heart and respiration beginning ten minutes after transection of the aorta, showing the onset of periodic respiration. *B*, records from the same fish beginning fifty-three minutes after transection, showing dyspneic periodicity giving way to weak, slow respiration.

more regular rhythm. The heart rate showed some irregular slowing but otherwise was generally more rapid, probably indicating a partial loss of vagus tone. In this fish the medullary centers remained reflexly functional for sixty minutes without a blood supply.

Discussion

The results reported above confirm Schoenlein (1895) insofar as an increase in the rate of flow of water to the gills caused an increase in respiratory rate. Springer (1928), working on *Mustelus canis* and *Squalus acanthias*, also reported that "the stronger the current the greater the rate of respiration." The marked, though rough, synchronism between the respiratory rate and the rate of the heart on altering the flow of water is difficult to understand, especially since the heart rate was changed about twice as much as the respiratory rate. It suggests a close nervous association between the respective medullary centers, although Lutz (1929) has found that each center may act independently. Inasmuch as accelerator nerves to the heart have not been demonstrated, the increase in heart rate accompanying increased respiration is probably due to inhibition of vagal tone. The vagus is known, under experimental conditions at least, to exert a continued influence on the heart (Lutz, 1930). Since *Scyllium* takes in water through the mouth on inspiration (Baglioni, 1907–08), a mechanism for increased respiration and heart rate during rapid swimming is clear.

That peripheral stimulation by water, however, is not necessary for continued regular respiration is obvious from Fig. 3. The inhibition on stopping the flow lasted only twelve seconds in this case, and ten seconds in the case illustrated in Fig. 4. This inhibition appears to be a transient reflex due to stimulation by collapse of the pharynx on stopping the flow of water. It occurred immediately, rather than after the water had been pressed out by several gill movements and air had entered, as suggested by Schoenlein (1895). Moreover, after the water had been expressed, a regular rhythm, rather than inhibition, was set up. It is apparent that, just as in the higher vertebrates, the rate of respiration is easily subject to peripheral control, but that a slow regular rhythm will continue without the usual peripheral stimulus.

The continuance of regular respiratory movements after transection of the aorta indicates an automaticity of the respiratory center without a blood supply. A rhythmic discharge from the cord in higher vertebrates has been found to occur under asphyxial conditions by Langendorff (1887) and by Brown-Séquard (1893), but the result-

ing movements are not considered to be true coördinated respiratory processes. In several teleosts Kolff (1908) noted the continuance of breathing movements for fifteen minutes after the heart had been removed. In the case of *Scyllium* with a transected aorta the regular rhythm which occurs at first, the onset of periodicity, and the subsequent dyspnea parallel the results of Baglioni (1907–08) with intact fishes in evacuated sea water. The usual explanation of periodicity involves alternate changes in the gas content of the blood flowing through the respiratory center. However, it appears that a periodic rhythmic discharge may occur without a blood supply, and therefore the cause for the periodicity must lie primarily within the nerve cells of the center.

Since it is impossible in the fish to cut the afferent fibers from the gills without destroying the motor pathways for respiration, conclusive proof of the automaticity of the respiratory center will be difficult to obtain. However, since rhythmic respiration continued after the water was stopped and the blood supply to the medulla interrupted, one may infer that, in fishes as in mammals, the respiratory center is autonomous. Furthermore, as in mammals, it is influenced by the gas content of the blood and by the peripheral stimulation.

Summary

1. The respiratory and cardiac responses to stopping and starting the water supply to the gills, to increasing and decreasing the rate of flow, and to complete interruption of the blood supply to the medulla of *Scyllium canicula* were recorded.

2. Sudden stopping and starting or increasing and decreasing the water supply evoked brief respiratory inhibition and sometimes ejection reflexes.

3. An increase in the rate of flow of sea water to the gills caused an increase in respiration and heart rates. A decrease caused the reverse. Too great an increase, however, evoked irregular inhibition.

4. Stopping the flow of water to the gills evoked brief inhibition of respiration followed by a regular rhythm at first, which sometimes became periodic and finally was interrupted by generalized muscular activity. The medullary centers remained reflexly functional for over three and one-half hours.

5. Interruption of the blood supply to the medulla when there was no water supply to the gills was followed by regular respiration at first, then periodicity and dyspnea. The medullary centers remained reflexly functional for over sixty minutes without a blood supply.

6. It is concluded that the respiratory center is autonomous, but that it is influenced by the gas content of the blood and by peripheral stimulation.

I wish to thank Dr. Reinhard Dohrn and Dr. Enrico Sereni for the many courtesies extended at the Zoölogical Station, Naples, and Edna B. Lutz for technical assistance.

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THE MEASUREMENT OF THE RESPIRATORY EXCHANGE OF AQUATIC ANIMALS

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INTRODUCTION

Recognition of the importance of studying the gas exchange of aquatic animals is attested to by the large number of papers which have appeared on this subject. The present article is the result of a series of investigations on the accuracy of various methods previously described for the measurement of this exchange and presents a method which has proven satisfactory for a rather wide variety of purposes.

METHODS OF MEASURING THE GAS EXCHANGE OF AQUATIC ANIMALS

There are three general types of methods of measuring the respiratory exchange of aquatic animals. The simplest method consists merely in determining the time rate of change in the gas contents of a closed vessel of water containing the animal under investigation. Details and applications of the method are given by Humboldt and Provençal (1809), Henze (1910*a*), Montuori (1913), Krogh (1916), McClendon (1917), Powers (1922), and Bruce (1926), among many others. The fact that oxygen is diminishing and carbon dioxide and nitrogenous excretions are accumulating in the water during the period of the determination renders this method unsatisfactory. Further objections will appear in the fourth section ("Method finally adopted . . .") of the present paper.

Applications of the Regnault (Regnault et Reiset, 1849) principle to aquatic respiration constitute the second general method. It has been used in various forms by Jolyet et Regnard (1877), Gréhant (1886), Zuntz (1901), Bounhiol (1905), and Gardner and Leetham (1914). The many complications and sources of error in this method have led to criticisms of it by Krogh (1916, p. 50) and Henze (1910*b*, 1927).

The third method for measuring the gas exchange of aquatic organisms involves a flowing water system. Briefly, a measured amount of water of known gas content is passed, in a given period of time, through a vessel containing the animal, and samples of the water

are collected and analysed after passing through this respiratory chamber. The application of this method has been almost entirely limited to special cases where a sort of artificial respiration is needed, such as in the case of narcotized fish, etc. Thus Winterstein (1908) used it to measure the strictly branchial respiration, while Ege and Krogh (1914) and Gaarder (1918) used it to determine the oxygen uptake of fishes under urethane narcosis. Hall's (1929) use of this method is open to criticism, as I have pointed out elsewhere (Keys, 1930*a*).

The Investigation of Methods in this Laboratory

Of the three general methods mentioned above, only the first and third were considered. The first method was subjected to a thorough trial in four different arrangements of apparatus. The test animals used in each case were marine fishes (*Fundulus parvipinnis*). A number of difficulties which seemed to be inherent in the method were found. It is not necessary to describe these experiments in detail; one particular source of difficulty was stratification in the gas content of the water in the respiratory vessel.[1]

Having taken into account as many of the sources of error as possible, the final test was made of attempting to duplicate results for the oxygen consumption of individual fishes by this method. In but few of these experiments was the oxygen consumption, as indicated by one determination, duplicated within 15 per cent by a second.

At this juncture experiments with the third method were begun. The apparatus arrangement which had been used by Ege and Krogh was used first. Various modifications of apparatus and technique were subsequently tried until the final method, which has been adopted as standard in this laboratory, was evolved.

It was found that the abstraction of the sample of the incoming water in the manner in which it was done by Ege and Krogh (see Krogh, 1916, p. 51) and, so far as I know, by all others who have used the flowing water method, not infrequently leads to serious errors due to stratification of the water through the respiratory chamber. The most serious drawback to the method of Ege and Krogh was the use of narcotics. Fortunately, however, it was found that the normal, unanesthetized animal could be used without difficulty. A large number of fishes, representing six different species, were observed in small respiratory chambers similar to those used by Gaarder and in every case the fish became perfectly quiet in a few minutes and remained so for many hours so long as a sufficient flow of water was provided.

[1] In some of these earlier experiments I had the assistance of Mr. Rolland Main, whose help is gratefully acknowledged.

Method Finally Adopted for Fishes and Other Macroscopic Aquatic Animals

Figure 1 shows the apparatus used for experiments on the gas exchange of fishes. The respiratory chambers *A* and *B* each contain a single fish and are chosen to be of such size that, although almost the whole of the water passing through the chamber comes into contact with

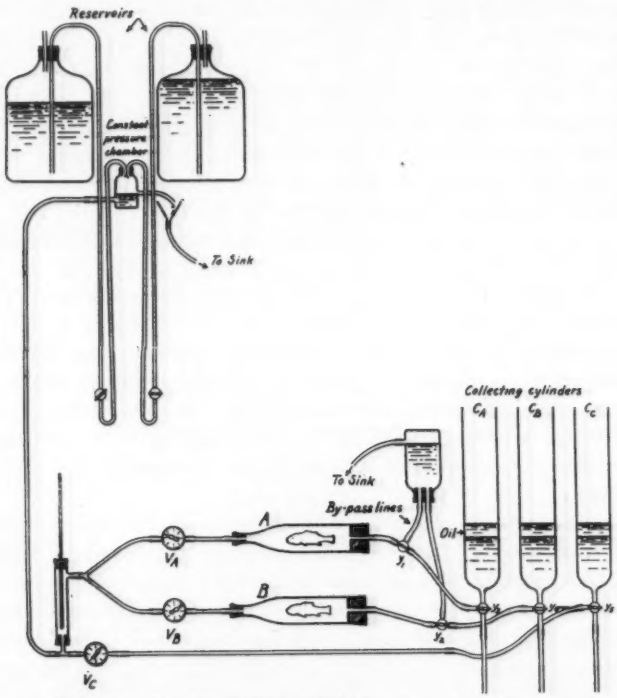

Fig. 1. Apparatus for determining metabolic rates of aquatic animals. The proportionate sizes shown are for the arrangement of the apparatus for studies of the respiratory exchange of small fishes such as *Fundulus*.

A, B, respiratory chambers; $Y_{1\text{-}4}$, 3-way stopcocks; $V_{A,\ B,\ C}$, needle valves.

the animal and is available to it for respiration, the fish is not ordinarily in contact with the walls of the chamber and is free to move about to some extent. If the fish is not disturbed after it is placed in the chamber, and if the current of water is properly adjusted at a constant flow, the fish quickly assumes a position facing the incoming stream and thereafter almost perfect quiescence is the rule, the only visible mus-

cular activity being the respiratory movements of the operculum and the leisurely motion of the pectoral fins which serves to keep the fish oriented in the current.

The collecting cylinders C_A and C_B receive the whole of the water which passes through respiratory chambers A and B respectively except when the water is by-passed through the by-pass lines. The by-pass chamber is so designed that it gives approximately the same back-pressure as the collecting cylinders when they are in use and, accordingly, with a given setting of the needle valves V_A, V_B, and V_C, the rate of flow through the system is the same, irrespective of whether the water is being collected for analysis or by-passed. The constant pressure chamber insures the head pressure remaining the same, no matter what the amount of water in the reservoirs. If the collecting cylinders are immovably fixed in position, the back pressure will increase as the cylinders fill, with a resulting decrease in the rate of flow; this can be taken care of by suspending them by long springs or by manually adjusting their level from time to time during each collection.

Collecting cylinder C_C receives the control water for the determination of the gas content of the water before it is respired by the fishes. The possibility of error due to stratification in the reservoirs is thus eliminated by a constant fraction of all the incoming water being diverted to the control. If stratification persists in the collecting cylinders it is easily broken up by gentle shaking or stirring of these last; this, of course, does not disturb the animals in the respiratory chambers.

It will be noted that no provision is shown for maintaining constant temperature. To secure the best results the whole apparatus should be mounted in a constant temperature room. Failing this, the tubing connecting the needle valves with the respiratory chambers should be lengthened into long coils and these last, with the attached respiratory chambers, should be suspended in a constant temperature water bath. In this case the thermometer chamber should be placed inside the bath at the end of one of the coils.

All water for gas analysis should be collected under at least three centimeters of the best quality saturated-hydrocarbon mineral oil. The gas content of the water is readily determined by standard methods, although the determination of carbon dioxide in sea water presents some difficulties. For oxygen the best method is undoubtedly the Winkler (1888) iodometric volumetric method. This method is given in detail in the American Public Health Association manual (3rd edition, 1917), by Treadwell and Hall (1928), and by Sutton (11th

edition, 1924). The determination of dissolved oxygen in sea water is fully described by Jacobsen (1921). The determination of carbon dioxide in the water may be made by the Van Slyke manometric method which will be found in the articles by Van Slyke and Stadie (1921), Van Slyke (1927), and Van Slyke and Sendroy (1927). An alternative method for determining CO_2 in sea water and brackish waters which utilizes pH determinations is given by Bruce (1924).

It should be pointed out that simple modifications, such as in the size of various parts of the apparatus, enable the flowing water method, as presented in this paper, to be used for very small animals. A correspondingly small volume of water must be used, and in this case the Van Slyke apparatus may be used for both oxygen and CO_2 in water volumes as small as one or two milliliters. The micro-Winkler method for oxygen (Thompson and Miller, 1928, and Snoke, 1929) may be used with samples as small as five milliliters. In all determinations, of course, duplicate analyses should be made.

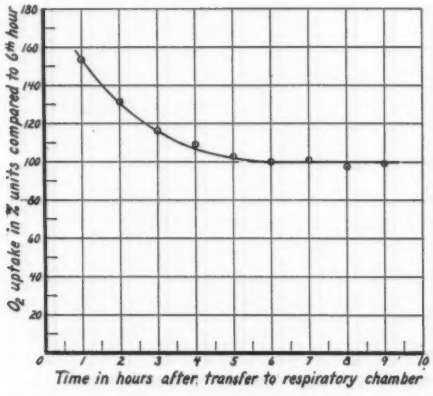

FIG. 2. Serial determinations of the oxygen uptake of *Fundulus parvipinnis*, showing initially high values and the basal or standard rate of exchange attained after several hours. Average curve for ten individuals.

One of the most important considerations in determinations of the gas exchange of the fishes is the time element. In all experiments reported in the literature the determination was begun as soon as the animal had ceased violent muscular activity, although in some cases even this precaution was omitted. Usually a single determination was made, the period of this determination varying in different cases from ten minutes to three or four hours; in a very few cases a longer period was

used. With this in mind I wish to call attention to Fig. 2. The points in this graph were obtained from serial determinations on ten different specimens of *Fundulus parvipinnis*. In order to bring out the general trend in the oxygen consumption of these fishes from hour to hour after transfer to the respiratory chamber, I have reduced all the data to percentage values, taking the oxygen consumption at six hours as the point of 100 per cent. It is clear that a constant rate of oxygen consumption is attained about five or six hours after transfer of the fishes to the respiratory chambers, and that determinations made before this time had elapsed would have overestimated the standard metabolism.

The appearance of the graph for *Fundulus parvipinnis* is characteristic of all the species of fishes which have been studied in this laboratory.[2]

Figure 3 shows an unusually complete series of determinations on a single specimen of *Girella nigricans* and depicts the constancy of results which may be obtained by the use of the method described in this paper.[3] I wish to make it clear that in all cases the fishes were very quiet after the first quarter or half hour in the respiratory chambers. In spite of this fact, the gas exchange did not reach a constant level until from four to six hours. In several hundred of these serial determinations this was found to be invariably the case. The results of a series of determinations on a specimen of *Clinocottus analis* are shown in Fig. 4.

These same general results were obtained in large as well as in small respiratory chambers and were found when the fishes were in total darkness as well as in ordinary daylight. They point to the conclusion that, in order to be sure that the determinations represent "standard" or anything like "basal" metabolism, it is necessary to wait five or six hours after the fish has been placed in the apparatus before beginning the final determinations. These findings throw doubt on the validity of any method in which the period of the determination of the gas exchange begins shortly after the transfer (and handling incident to this) of the animal to the vessel which is used for the experiment.

The procedure recommended in beginning gas exchange experiments on an animal form which has not previously been investigated, is to make the determinations serially, which is very simple with the apparatus described in this paper, until constant gas exchange is reached. Experiments in this laboratory indicate that, in the case of

[2] These species are *Girella nigricans, Fundulus parvipinnis, Clinocottus analis, Leptocottus armatus.*

[3] I was fortunate in having the collaboration of Mr. N. A. Wells in the application of the method to *Girella* and to *Clinocottus analis.*

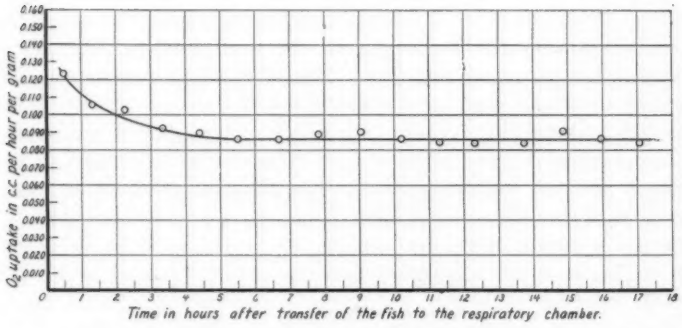

FIG. 3. Serial determinations of O_2 uptake of *Girella nigricans*, fish no. G_4, weight 40 grams. Three days starvation. Temperature 16.7° C.

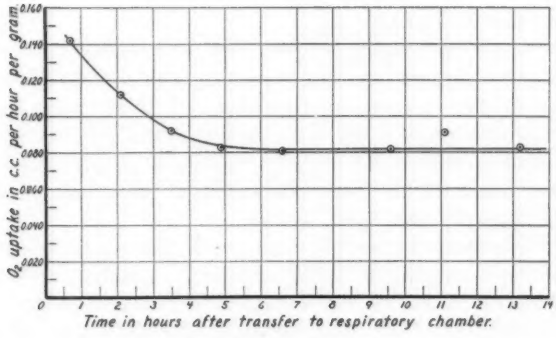

FIG. 4. Serial determinations of O_2 uptake of *Clinocottus analis*, fish no. C_3, weight 37 grams. Temperature 20.0° C.

most fishes, it is perfectly safe simply to transfer the animals to the apparatus, allow the system to run (through the by-pass lines) for six hours, and then make two or more determinations covering, let us say, about two hours as a minimum. If these determinations check reasonably well (as is ordinarily the case), it may be assumed that the results (or the mean of the results) represent standard metabolism.

The characteristic appearance of the time graph of the gas exchange demands some comment. The elevated gas exchange which persists for a matter of some hours is not due to muscular activity, at least no such activity is visible. It seems certain that at least part of it is due to the payment of an oxygen debt contracted by the fish in its struggle incident to being placed in the apparatus.[4] Another possible explanation for the phenomenon might be found in the fact that, whereas a certain minimum stimulus is necessary to bring about a nervous impulse and consequent muscular action ("all or none" principle), sub-minimal stimuli will cause a marked increase in the gas exchange of nervous tissue at least (Winterstein and Hirschberg, 1927).

I have already indicated how, by making serial observations, it is a simple matter to demonstrate that the method developed here is capable of yielding very consistent results. In a number of cases determinations of the oxygen uptake of certain fishes were repeated after a lapse of from two or eight days. The results of these experiments are summarized in Table I.

The average discrepancy between first and second determinations is 2.8 per cent. In each case the oxygen uptakes in the table represent the average of two or more determinations. I should warn the reader that such good results are not to be expected until the investigator has had considerable experience with the technique. Good results require unremitting vigilance in the maintenance of constant physical and physiological conditions.

It will be noted that in Fig. 1 there are two respiratory chambers shown. This is a highly desirable feature as it enables the investigator to compare two animals, even though it is not possible to maintain standard conditions. Systematic errors in the determinations are fre-

[4] The period of payment of oxygen debt is usually not nearly so prolonged in mammals (Hill and Lupton, 1922; Campbell, Douglas and Hobson, 1920; Krogh and Lindhard, 1920) as is indicated in the experiments on fishes but, as Barcroft (1925) points out, the duration of the debt is directly related to the adequacy of the blood supply, and we know (Krogh, 1929) that the capillary blood supply to the muscles of fishes is meagre compared to that of the warm-blooded animals. We should expect the payment of the oxygen debt of any cold-blooded animal to be slower than in a warm-blooded animal in the same way that all the vital processes proceed at a relatively slow rate in the cold-blooded animals.

quently detected if two animals are used in this way. Of course, if the experimenter desires, three, or even more, respiratory chambers with their respective collecting cylinders, valves, etc., may be used, but the services of a number of assistants will be found to be necessary if this is done.

TABLE I

Species	Fish No.	Weight	Date	Temperature	O_2 uptake per hour
		grams		°C.	*grams*
Fundulus	M_{35}	4.3	12/26/28	20.0	0.207
"	"	"	12/31/28	"	0.214
"	M_{43}	9.1	6/25/29	"	0.161
"	"	"	7/ 2/29	"	0.159
"	M_{44}	4.5	6/25/29	"	0.209
"	"	"	7/ 2/29	"	0.202
"	M_{50}	7.3	6/28/29	"	0.194
"	"	"	6/30/29	"	0.208
"	M_{51}	4.4	6/28/29	"	0.280
"	"	"	6/20/29	"	0.281
Girella	A_7	257	12/ 5/29	20.0	0.0615
"	"	"	12/10/29	"	0.0623
"	A_9	210	12/12/29	18.0	0.0586
"	"	"	12/20/29	"	0.0607

There are a number of precautions which should be taken. The water used should always be filtered and well aërated before use, but should never be supersaturated. The whole system should frequently be tested for leaks and should never be used with air bubbles trapped in any part of the apparatus. The animals used should always be starved long enough before the determination to insure that the digestive tract is empty at the time of the experiment.

Applications of the method described here to particular problems involving a study of the gas exchange of aquatic animals are given in a paper by the author (Keys, 1930*b*) now in press, as well as in other papers in the course of preparation at the Scripps Institution.

CALCULATION OF RESULTS IN RESPIRATION EXPERIMENTS

The calculations for the determination of oxygen and carbon dioxide by the Van Slyke manometric apparatus are given in the papers of Van Slyke already cited. The calculations for oxygen by the Winkler method are given in the references on this method listed above.

Tables of the normal saturation values of water with respect to oxygen are given by Fox (1907), Jacobsen (1921), and Harvey (1928). Fox's data (given also by Harvey) were obtained by equilibrating water of various salinities with atmospheric air at different temperatures. Jacobsen's data are for air minus water vapor and carbon dioxide. Salinity may be converted into chlorinity by the use of Knudsen's formula

$$Cl = \frac{\text{salinity} - 0.030}{1.8050}$$

where *Cl* is the chlorine equivalent of the chlorine, bromine and iodine in the sea water, and salinity is the total weight of salts (carbonates as oxides and organic matter ignited) in grams per 1000 grams of sea water.

Summary

The methods previously used for the measurements of the gas exchange of aquatic animals are discussed. Of the three general methods, two were subjected to careful investigation in experiments on marine fishes.

The method finally developed for the study of aquatic respiration is described in detail and typical results obtained by its use are given. It is shown that the method yields consistent results which may be accepted as measures of standard metabolism.

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RATE OF HISTOLYSIS OF ANURAN TAIL SKIN AND MUSCLE DURING METAMORPHOSIS

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The factors responsible for the atrophy of the larval anuran's tail will no doubt require considerable further work before a complete and adequate explanation can be derived. The main results and conclusions reached to date may be briefly reviewed as follows: Barfurth (1887) first suggested that the growing urostyle occluded the adjacent dorsal aorta and so cut off the blood supply to the tail. Mercier (1906) later found phagocytes in the muscle masses of the tail which contained carmine granules previously injected into the dorsal lymph sac. This indicated that the occlusion of the aorta due to urostyle growth must be only a partial one. Bataillon (1891) studied the blood vessels of normal and atrophying tails and described a change in vascular distribution (notably of the capillaries) in the latter. More recently, Morse (1918) and Bradley (1922) have reiterated Barfurth's explanation and suggest that the reduced blood supply to the tail enables the CO_2 and various acid metabolites to accumulate. The result is the activation of the autolytic enzymes with consequent histolysis and general atrophy of the tail. Morse also suggested that a possible lowering of the blood pH might be the fundamental causative factor involved.

A new conception regarding the cause of anuran tail atrophy was evolved through the work of Helff (1928), which demonstrated that larvæ in which the anlagen of the urostyles had been extirpated exhibited typical tail atrophy during subsequent metamorphosis. The growth of the urostyle and the partial occlusion of the dorsal aorta could not, therefore, be looked upon as the fundamental factors inducing tail atrophy. Simultaneously, with this finding, Lindeman (1929) showed that tail skin transplanted autoplastically to the backs of larvæ underwent rapid and characteristic histolysis at the same stage of metamorphosis at which the integument of the tail normally degenerates. Back-skin grafts, previously transplanted to the tail, failed to undergo degeneration during larval involution. Helff and Clausen (1929) were likewise able to demonstrate practically the same results using tail-and back-muscle transplantations. It was found

in this connection that although back muscle transplanted to the tail underwent 54 per cent reduction in volume due to atrophy during metamorphosis, similar sized grafts of tail muscle previously transplanted to the back were completely obliterated during the same time interval. The above results on skin and muscle transplantations suggested the probability that the various tissues of the tail are especially susceptible to histolytic reactions and that such reactions are the result, perhaps, of the presence of specific histolytic substances in the blood or of a general lowering of the pH of the latter during larval transformation.

During the course of Lindeman's (1929) work, the writer's attention was called to the fact that skin grafts placed near the anterior-posterior center of the larval tail were found to undergo a gradual migration towards the base of the tail. It was observed, however, that while the distance between the graft and tip of tail remained practically unchanged, the space between the base of the tail and the graft was constantly reduced as tail atrophy proceeded. Schubert (1926), during the course of work involving the transplantation of hind-limb buds to the tail, noted that the developing hind-limb transplants appeared to move progressively anterior as histolysis of the tail occurred during larval involution. The results of Lindeman and of Schubert both suggested the possibility that the anterior regions of the larval tail undergo histolysis at a considerably greater rate than is true of more posterior regions. The present paper records the results of experiments which were devised to test out the veracity of this assumption. In general the experiments consisted of the autoplastic, reciprocal transplantation of muscle and skin grafts of the back and tail, the transplants of tissue from the latter structure being taken from various longitudinal levels.

The experimental part of the present work was completed at the Iowa Lakeside Laboratory, Milford, Iowa, during the summer of 1929. The writer is greatly indebted to Dr. O. M. Helff for suggesting the problem and for his valuable suggestions and criticisms during the course of the work.

Material and Methods

The stock used for all operations were large *Rana pipiens*' larvæ, obtained from small ponds in the vicinity of Spirit Lake, Iowa, during the months of June and July, 1929. The animals when taken to the laboratory, were placed in large battery jars and fed fresh *Spirogyra* each day. The individuals selected from this stock ranged from 60 to 100 mm. in body length, with hind limbs between 4 and 15 mm. in

length. These animals were normal larvæ and remained unchanged in the laboratory as typical larvæ for at least one week following the operations.

All animals used for transplantation purposes were anæsthetized in a 0.05 per cent aqueous solution of chloretone. The time required for anæsthetization by this method varied from five to ten minutes, depending somewhat on the size of the animals used and the temperature of the solution itself. The anæsthetized animals usually remained inactive for a period varying from one to two hours, thus giving sufficient time to allow the muscle grafts and the cut edges of the integumentary transplants to adhere properly.

The technique used in making the muscle transplantations was briefly as follows: A flap of the integument was first laid back and a small piece of muscle removed and placed in a few drops of lymph in a watch crystal. This piece was then accurately shaped under a dissecting microscope so that a cube of tissue, eight to twelve cu. mm. in volume, was prepared for transplantation. In transplanting, a flap of integument was likewise laid back from the region to receive the transplant, the underlying musculature lightly scraped, and the cubical muscle transplant then placed on this surface. The flaps of integument were now replaced and the animal placed in shallow water, with the operated areas exposed to the air so as to hasten the adherence of the cut edges of the skin flaps. All operations were autoplastic and reciprocal, *i.e.*, from tail to back and back to tail of the same individual.

The usual method of skin transplantation was also employed. Following the anæsthetization, integument from four regions of the tail was removed and each portion of integument placed in a watch crystal. The four pieces of integument were now shaped in the form of rectangles so that they corresponded in length and width one with another. The integument from four adjoining medial regions of the back was next removed and similarly shaped. The tail-skin grafts were finally transplanted to the wound areas on the back, and the back-skin grafts transplanted to the denuded areas of the tail. Following the usual exposure of the grafted areas to the air, the individuals were submerged in water to recover from the effects of the anæsthetization. Upon recovery, the operated individuals were placed in individual battery jars and maintained under constant laboratory conditions. Daily observations were made on all operated animals in regard to the process of healing, histolytic condition of the transplants, changes in form and size of the grafts, and the onset and progress of metamorphosis.

Results

1. *Transplantation of Tail and Back Muscle*

The purpose in making autoplastic transplantations of muscle secured from the anterior, posterior, and two intermediate regions of the tail to the backs of larvæ was, primarily, to see whether or not muscle tissue from one region of the tail would undergo normal atrophy before that of other tail regions. The tail-muscle tissue to be transplanted was obtained from the side of the tail at either a posterior, anterior, or from one of two intermediate areas (text figure 1, areas *A*, *B*, *C*, *D*).

The muscle, after being carefully shaped and measured as previously described, was then transplanted, autoplastically, to a position slightly to one side of the mid-dorsal line on the back. (Text figure 1,

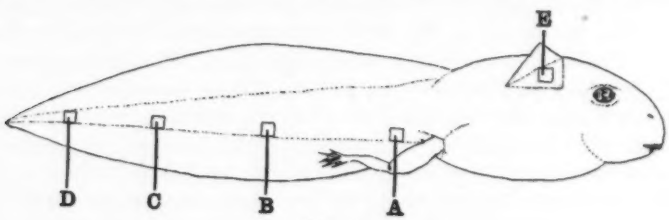


Fig. 1

E.) This position was selected to insure adequate contact of the graft with the underlying back muscle, since musculature of the mid-dorsal region of the back is very thin and rather poorly vascularized. The reciprocal transplantation of back muscle to the tail of the same individual was made in a similar manner for the purpose of showing possible differences in histolytic influence of the two foreign host regions on their respective transplants. The back muscle transplants, however, were always obtained from one region of the back and transplanted to the middle of the tail.

One hundred and twenty-one successful reciprocal transplantations of tail and back muscle were obtained. The percentage of abnormal grafting proved to be less than one per cent. Table I represents the essential volume reduction data, due to histolysis during metamorphosis, for the four series of muscle transplantations. Series *A* represents those individuals in which the tail-muscle transplant was removed from the most anterior region of the tail; series *B* and *C* representing cases in which the muscle transplants were obtained from the intermediate tail regions; while series *D* were individuals whose muscle

transplants were taken from a posterior region of the tail. The series therefore correspond in lettering to the operative areas *A*, *B*, *C*, and *D* as indicated in text figure 1. The various percentage reductions are based on the average volume reductions recorded at eleven-, sixteen-, and twenty-two day intervals following the operative procedures. The histological appearance of the grafts, sixteen days following transplantation, seems to compare favorably with that as described by Helff and Clausen (1929) for grafts examined fifteen days following transplantation.

TABLE I

Histolysis of Tail- and Back-muscle Transplants during Metamorphosis

Series	No. of Cases	Type of Transplantation	Average Volume Reduction		
			11 days	16 days	22 days
			per cent	*per cent*	*per cent*
A.....	31	Tail to back	47	79	98
		Back to tail	20	30	30
B.....	27	Tail to back	44	77	94
		Back to tail	20	35	35
C.....	31	Tail to back	40	70	92
		Back to tail	20	38	40
D.....	32	Tail to back	32	65	88
		Back to tail	20	38	38

An analysis of the data as represented in Table I will show that the tail-muscle transplants are markedly more susceptible to histolysis than is true of the back-muscle transplants. This is of even more interest when we consider that the latter transplants were surrounded by the atrophying musculature of the tail, while the tail-muscle transplants were in contact with back musculature which undergoes a lesser degree of resorption. Of primary interest, however, are the results of the muscle transplants obtained from different regions of the tail. Examination of the data will show that at all time intervals examined, the more anterior muscle grafts underwent a greater average percentage of atrophy than was true of more posterior muscle grafts. This would indicate that the anterior musculature of the tail is more susceptible to histolytic influences than is true of more posteriorly located muscle.

2. *Reciprocal Tail and Back Integumentary Transplantations*

The purpose of transplanting integument from various regions of the tail to the back was mainly to determine whether or not more an-

terior tail integument would exhibit a more rapid histolysis as compared with integument obtained further posterior on the tail. The reciprocal transplantation of back integument to the tail was primarily to see whether or not such integument would fail to undergo resorption during larval involution, as previously pictured and stated by Lindeman (1929).

The integumentary tail grafts were obtained from four regions of the tail (1, 2, 3, 4, text figure 2). These grafts will henceforth be

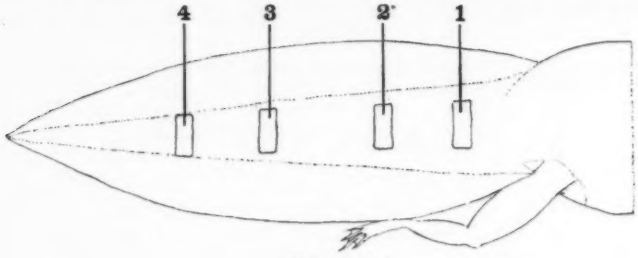


FIG. 2

spoken of as tail-skin transplants number 1, 2, 3, and 4, respectively. The integument, after being removed, was carefully shaped to form 2 × 7 mm. rectangles in order that the area reduction of this standard-sized graft could be accurately determined following subsequent transplantation and larval involution. Pieces of integument were next

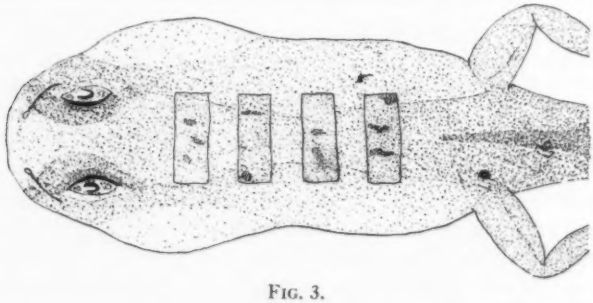


FIG. 3.

removed from four separate regions on the back and likewise shaped to conform to a 2 × 7 mm. rectangle. The tail-skin transplants were now grafted to the wound areas left by the removal of the back-skin transplants (text figure 3). Four types of orientation regarding the linear sequence of transplanting the tail-skin were made. In some cases tail-skin transplant number 1 was grafted to the most anterior

of the wound areas of the back, while tail-skin transplants number 2, 3, and 4 were grafted to respective posterior wound areas. This arrangement is spoken of as the 1-2-3-4 sequence. Where the reverse arrangement was made during transplantation (tail-skin transplant number 1 to the most posterior wound area, tail-skin transplant number 2 to the adjacent anterior wound area, etc.) the arrangement is spoken of as the 4-3-2-1 sequence. In addition to the above two arrangements of the four transplants, the sequences of 3-4-1-2, and 2-1-4-3 were also employed. The four back-skin transplants were likewise grafted to the wound areas on the tail using the same four sequences in the linear arrangement of the grafts.

Subsequent observation involved the measuring of the linear dimensions of the eight integumentary grafts of each animal at eleven- and sixteen-day intervals following the transplantation date. In this manner a fairly accurate estimate of the histolytic rate of the various transplants could be determined as metamorphosis took place. The essential data for the various series are represented in Table II. The

TABLE II

Histolysis of Tail and Back Integumentary Transplants during Metamorphosis

Series	Type of Transplantation	Average Surface Area Reduction							
		8th Day Observation				16th Day Observation			
		Tail-skin Transplant Number *				Tail-skin Transplant Number *			
		1	2	3	4	1	2	3	4
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
AY (1–2–3–4) 22 cases	Tail to back	40	30	0	0	60	45	20	0
	Back to tail	0	0	0	0	20	15	0	0
BY (4–3–2–1) 24 cases	Tail to back	25	25	0	0	50	45	0	0
	Back to tail	0	0	0	0	20	10	0	0
CY (3–4–1–2) 20 cases	Tail to back	25	10	0	0	60	50	25	0
	Back to tail	0	0	0	0	30	15	5	0
DY (2–1–4–3) 22 cases	Tail to back	40	30	5	0	70	50	30	0
	Back to tail	0	0	0	0	35	20	0	0

* The numbers 1, 2, 3, and 4, as they refer to the *back to tail* transplantations, are used to indicate the respective wound areas of the tail to which the grafts were transplanted.

data consist of the results of observation on the eight transplants, each, of eighty-eight larvæ. The most significant finding as evidenced by these results is that tail-skin transplant number 1 always showed a comparatively greater histolytic rate, while tail-skin transplants number 2, 3, and 4 showed progressively lesser speeds of atrophy. This gradient in the histolytic rate was found to hold true regardless of the linear sequence in which the transplants had been placed on the back. In other words, the particular location of any one transplant on the back was of no consequence to the rate of histolysis occurring. This point may be more easily understood by reference to Figs. 1, 2, 3, and 4, Plate 1. Further measurements of the tail-skin transplants at a time when transplant number 1 had been entirely obliterated, showed that transplants number 2, 3, and 4 had been reduced on the average but 70, 50, and 20 per cent respectively, in surface area. The difference in histolytic rate of the four transplants at the sixteen-day observation period is quite evident from histological evidence as pictured in Fig. 5, Plate 1.

FIGS. 1, 2, 3, 4. Illustrating the appearance of the four tail-skin transplants autoplastically transplanted to the back. The sketches were made at a time when tail-skin transplant number 1 was considerably histolyzed. In each figure transplant number 4 is still unchanged in external appearance and size and represents the size of all four tail-skin transplants at the time of operation. 1, 2, 3, and 4, tail-skin transplants obtained from regions 1, 2, 3, and 4, respectively, of the tail; *BI*, integument of the belly.

FIG. 1. Transplantation sequence 1–2–3–4.

FIG. 2. Transplantation sequence 4–3–2–1.

FIG. 3. Transplantation sequence 3–4–1–2.

FIG. 4. Transplantation sequence 2–1–4–3.

FIG. 5. Histological section through back integument including the four tail-skin transplants at the stage illustrated in Fig. 1. 1, 2, 3, and 4, portions of section composed of the respective four tail-skin transplants showing degrees of histolysis corresponding to the macroscopic reduction in size; *E*, epidermis; *SS*, stratum spongiosum; *SC*, stratum compactum; *CT*, subcutaneous connective tissue.

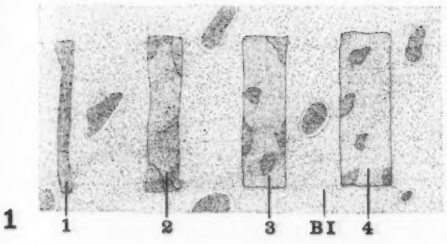
FIG. 6. Section through tail-skin graft on the belly and adjacent belly-skin as illustrated in FIG. 8, *TM*. The tail-muscle transplant is omitted in the figure. *BS*, belly-skin; *TG*, tail-skin graft; *x*, point of connection between belly-skin and tail-skin transplant.

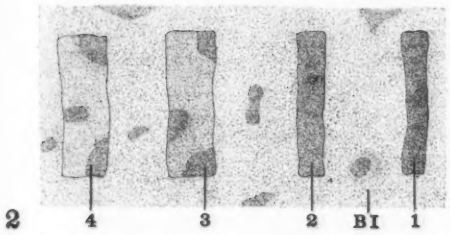
FIG. 7. Section through tail-skin graft on the belly and adjacent belly-skin as illustrated in Fig. 8, *BM*. The back-muscle transplant is omitted in the figure. Lettering the same as in Fig. 6.

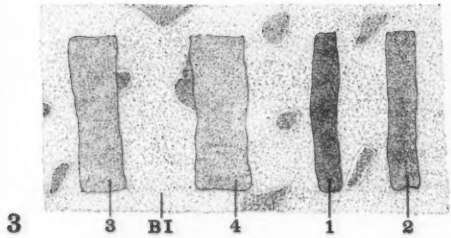
FIG. 8. Appearance of tail-skin grafts on the belly under which muscle transplants had been inserted four days previous. *TM*, graft (under which tail-muscle transplant had been inserted), showing decided macroscopic signs of histolysis; *BM*, graft (under which back-muscle transplant had been inserted), showing only slight macroscopic signs of histolysis; *BS*, belly skin.

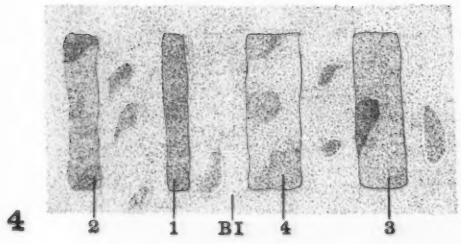
FIG. 9. The same grafts as illustrated in Fig. 8 eleven days later. Note excessive reduction in size of graft over tail-muscle transplant as compared with size of graft over back-muscle transplant.

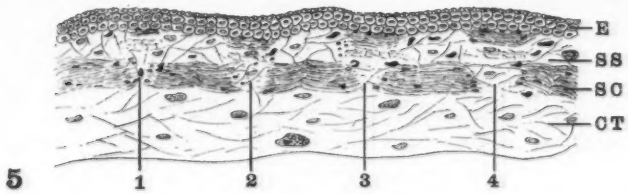
PLATE I

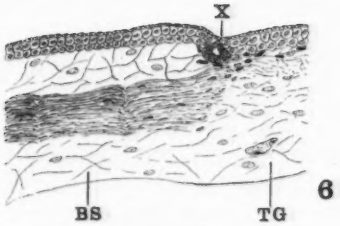

1
1
2
3
BI
4

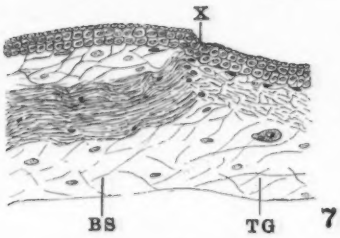

2
4
3
2
BI
1

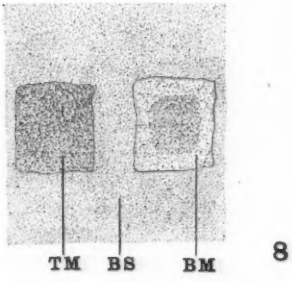

3
3
BI
4
1
2

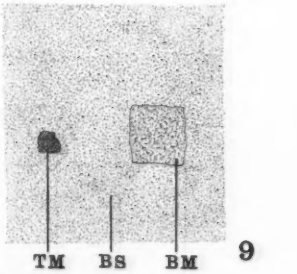

4
2
1
BI
4
3


5
1
2
3
4
E
SS
SC
CT


X
BS
TG
6


X
BS
TG
7


TM
BS
BM
8


TM
BS
BM
9

The four back-skin grafts transplanted to the tail proved to be remarkably resistant to histolysis during the early stages of tail atrophy. This confirms the findings of Lindeman (1929). All of the four back-skin transplants appeared to be equally resistant, but it was noted that as more pronounced atrophy of the tail ensued (see Table II, 16-day observation), the grafts that had been transplanted to the more anterior regions of the tail underwent a certain degree of histolysis, while those previously placed on wound area number 4 never gave evidence of degeneration. It would appear, therefore, that the comparatively greater rate of muscular histolysis in the anterior regions of the tail is capable, eventually, of inducing even back skin to degenerate.

3. *Effect of Atrophying Muscle on Histolysis of Tail Integument*

It was shown by Helff (1926) that atrophying tail muscle is capable of inducing histolysis in opercular skin grafts when transplanted beneath the latter. More recently Helff and Clausen (1929) have shown that tail muscle grafts undergo approximately a 62 per cent greater atrophy during metamorphosis when transplanted to the back than is true of back muscle transplanted from one side of the back to the other. It therefore seemed likely that the normally atrophying musculature of the tail may function to facilitate or even to accelerate the histolysis of tail integument during involution. Experiments were consequently devised to test out this assumption.

The operations performed were briefly as follows: Two grafts of integument (4 mm. square) were removed one from each side of the middle region of the tail of normal larvæ. These were then transplanted, autoplastically, one on either side of the mid-ventral line of the belly and the grafts allowed to heal firmly to the surrounding integument. Three days later a cube of back muscle (1.5 mm. square) was removed from the same larva and transplanted beneath one of the integumentary grafts. At the same time a similar sized piece of tail muscle was removed from the same larva and inserted beneath the other integumentary graft. Forty larvæ were so operated on in all. The larvæ were now allowed to metamorphose in order that the effects of the two types of muscle transplants on the tail-skin grafts could be determined.

The first signs of normal tail atrophy of the operated larvæ were evident within one or two days following the transplantation of the integumentary and muscle grafts. Usually about three days following the operative procedure, distinct macroscopic signs of histolysis were

evident in the tail-skin graft underneath which tail muscle had been inserted. This consisted chiefly in a characteristic darkening of the entire surface of the integument (*TM*, Fig. 8, Plate 1). The other skin graft, underneath which back muscle had been inserted, appeared more normal, although histolysis as evidenced by a darker coloration of the skin was typical of a central area of the transplant directly over the muscle graft (*BM*, Fig. 8, Plate 1). Histological sections made at this stage through these two tail-skin grafts (Figs. 6 and 7, Plate 1) gave evidence that more pronounced signs of tissue disintegration were evident in the graft over the tail-muscle as compared with the graft over the back-muscle transplant. Following this period, both integumentary grafts underwent a progressive histolysis and consequent reduction in size. Fifteen days following transplantation, the relative appearances of the two grafts were approximately as represented in Fig. 9, Plate 1. By the time the integumentary graft in association with tail muscle had practically been obliterated, the other graft associated with back muscle was approximately but 50 per cent reduced in area. The results serve to emphasize, therefore, that atrophying tail muscle is decidedly more effective in hastening the histolysis of tail integument than is the case of atrophying back muscle. The further inference may be drawn that the musculature of the tail is normally favorable towards or may even accelerate the histolysis of the integument of the latter organ during metamorphosis.

Discussion

The argument might well be advanced that one would expect a highly differentiated tissue like muscle to undergo perhaps complete degeneration following transplantation even in normal non-metamorphosing larvæ. This, however, cannot be accepted as a possible explanation of the present results since Helff and Clausen (1929) transplanted various types of anuran muscle in non-metamorphosing larvæ and recorded an average reduction in volume of less than ten per cent over a period of three weeks. The reduction in volume of the muscle transplants as described in the present paper must therefore be considered due mainly to the operation of definite histolytic factors functional during larval involution.

The question of whether or not the atrophying musculature of the tail actually tends to accelerate the integumentary histolysis of the latter organ is an interesting one. The results of the present paper confirm those of Helff (1926) in that atrophying tail muscle is capable of inducing histolysis of certain integuments. The results of Lindeman (1929), regarding the resistance of back skin to histolysis when

transplanted to the tail and the total histolysis of tail-skin transplanted to the back, are also corroborated. Slight histolysis, it would seem, may be induced in back-skin grafts when transplanted to the anterior regions of the tail for a considerable period of time. Lindeman's transplants, it may be stated, were all made to the central or more posterior tail regions and hence corresponded to the results of the present work in which skin grafts had been transplanted to areas 3 or 4 as represented in text Fig. 2. The results of tail- and back-muscle transplantation beneath tail-skin grafts gave distinct evidence that although the integument of the tail is capable of undergoing complete histolysis during metamorphosis when transplanted to the back, its rate of histolysis is probably accelerated considerably when in contact with the atrophying musculature of the tail.

The results adequately support the contentions of Helff (1928), Helff and Clausen (1929), and Lindeman (1929) in that the growth of the urostyle cannot be looked upon as the fundamental cause of anuran tail atrophy, but that the various tissues of the tail are hereditarily specific and susceptible to histolytic agents probably transported through the blood stream. This latter generalization would seem to be particularly true for the integumentary and muscular elements of the larval structure. The present results finally indicate that the integument and musculature are not equally susceptible to histolysis at all levels of the tail, but conversely, appear to exhibit what might be called a susceptibility gradient in that anterior muscular and integumentary regions are more susceptible to histolysis than is true of more posterior regions. Granting that the present results have proved the existence of a susceptibility gradient to histolysis, it is of interest to inquire regarding the origin of this gradient. The different degrees of susceptibility must either be inherited during the embryonic formation and development of the various tissues of the tail or else acquired during the growth period of the larval organ. To date, there are no experimental results available to warrant an adequate explanation of this point.

Summary and Conclusions

Preliminary observations indicated that different regions of the larval anuran's tail undergo histolysis at different speeds.

1. Uniform-sized, tail-muscle grafts were secured from extreme anterior and posterior regions and from two intermediate areas and transplanted, autoplastically, to the backs of *Rana pipiens*' larvæ. During metamorphosis, anterior grafts invariably histolyzed with greater rapidity (10 to 25 per cent) as compared with posterior muscle grafts. Intermediate muscle grafts histolyzed at proportionate speeds.

2. Similar grafts of tail skin were autoplastically transplanted. Regardless of their location on the back, skin grafts from anterior tail regions always underwent histolysis at a greater speed and prior to grafts from more posterior regions. Total histolysis of anterior grafts was frequently observed at a time when posterior grafts presented less than 50 per cent disintegration.

3. The comparative histolytic influence of tail and back muscle was questioned by the transplantation of muscle beneath integumentary grafts previously made to the belly. Integumentary grafts over tail-muscle transplants invariably underwent approximately at least 50 per cent greater histolysis as compared with grafts over back-muscle transplants.

4. The conclusion is reached that anterior regions of the larval anuran's tail undergo histolysis with a greater speed and prior to more posterior regions. A definite anterio-posterior gradient may be said to exist in this regard, the cause of which, however, is still undetermined.

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THE INNERVATION OF THE HEART OF THE ELASMOBRANCH, *SCYLLIUM CANICULA*

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(From the Zoölogical Station, Naples, and the Physiological Laboratory of Boston University School of Medicine)

The elasmobranch fishes have certain features, both structural and physiological, which make them of interest from a comparative viewpoint. The well developed cranial parasympathetic system and the poorly developed sympathetic pointed out by Müller and Liljestrand (1918), the abundant chromaphil tissue quite separate from the interrenal tissue first described by Balfour (1878), the extraordinarily high urea content of the blood, the significance of which Baglioni (1906) has studied, the marked cardiac and respiratory inhibition and the absence of vaso-motor responses noted by Schoenlein and Willem (1894) and the inhibitory action of adrenaline on the heart first noted by Macdonald (1925) are some examples. An attempt, however, to find a detailed description of the vagus supply to the heart of *Scyllium canicula,* which has been the chief type used, was unsuccessful, although the course of the vagus in certain fishes has been described by Dogiel and Archangelsky (1906). In the pike (*Esox lucino*) these authors trace a ramus cardiacus from the visceral trunk to the sinus-auricle junction where a plexus is formed giving off fibers to the ventricle. In *Scyllium,* Marshall and Hurst (1905) state that the visceral branch of the vagus gives off cardiac nerves to the heart. In the dogfish, *Squalus acanthias,* Norris and Hughes (1920) describe the cardiac ramus as arising from the post-branchial branch of the fourth branchial division of the vagus (fifth branchial nerve). In an investigation of the cardio-inhibitory reflex and of the visceral afferent nervous pathway in *Scyllium* the writer found it necessary to examine the vagus supply to the heart in some detail. Incidentally some observations were made on the presence of vagal tone.

Bottazzi (1902) attempted to demonstrate an accelerator innervation of the heart of *Scyllium,* with the medulla separated from the spinal cord, by stimulating various sections of the cord and the first sympathetic ganglion, but obtained no acceleration in the rate. With the idea that possibly there were accelerator fibers entering with the vagus, he stimulated this nerve electrically with gradually increasing

strengths of stimulus, but got only inhibition. He did not perfuse the gills during the procedure. Müller and Liljestrand (1918), in a morphological study, could find no nerve fibers running to the heart from the first large sympathetic ganglion in elasmobranchs. Since Zwaardemaker (1925) claims to have found cardio-accelerator nerves in *Petromyzon* in which previously both Greene (1902) and Carlson (1904) had failed to demonstrate such nerves, the writer considered it worth while to repeat, with perfusion of the gills, Bottazzi's experiments, and to try certain other procedures which might possibly reveal the presence of an accelerator control of the heart.

Material and Methods

Specimens of *Scyllium canicula* averaging 300 grams were used. Gross dissections were made in living fishes with the brain destroyed anterior to the optic lobes and the spinal cord pithed posteriorly from various levels. Morphological observations were checked by stimulating the nerves in question both centrally and peripherally with a faradic current, or by cutting all nerves except the one to be examined and testing its function in reflex cardio-inhibition. In one case a complete dissection was stained with osmic acid. By a method described in a previous paper (Lutz, 1930) the heart and the respiratory rate were recorded.

A similar type of preparation, with certain parts of the nervous system destroyed and with the gills perfused, was used to investigate the possible existence of accelerator influence.

Results

In studying reflex cardio-inhibition it was at once obvious that cutting the cardiac root of the visceral branch of both vagi failed to prevent reflex cardiac arrest on stimulation of the skin and various other points. On the assumption that *Scyllium* might be similar to *Squalus* with respect to vagus innervation, the post-branchial root of the fourth branchial division (fifth branchial nerve) on each side was also cut. This procedure prevented the elicitation of reflex cardio-inhibition. Cutting either pair of roots alone did not prevent direct inhibition of the heart on stimulation of the nerve anterior to the fourth branchial division. All four nerves must be cut, or section of the main trunk be made anterior to the fourth branchial division in order to remove all vagus influence. A careful dissection was then made of many specimens and the two branches from each vagus were found to run to the Cuverian duct and there anastomose on the wall of this large

vein before passing to the heart. Weak faradic stimulation of the cut distal portion of each branch produced inhibition, and stimulation of the central end caused reflex cardio-inhibition provided any one of the branches remained intact.

Reflex cardiac and respiratory inhibition was obtained on mechanical stimulation of the dorsal surface of the ventricle either by pinching it lightly with the fine forceps or by sticking the epicardium with a sharp needle (Fig. 1, *A* and *B*). This was also obtained after the aorta had been transected. Reflex cardio-inhibition was also elicited on weak faradic stimulation of the ventricle in a fish with the spinal cord pithed posteriorly from the fifth vertebra and the hypobranchial nerve and all other spinal nerves of the region cut. All branches of the vagus except the post-branchial ramus of the fourth branchial division on each side were cut (Fig. 1, *C*). The cardio-inhibition was accompanied by

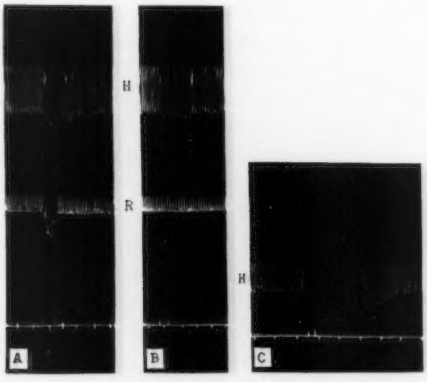

FIG. 1. Reflex cardiac and respiratory inhibition on stimulation of the dorsal surface of the ventricle of *Scyllium canicula* with the forebrain destroyed and the spinal cord pithed posteriorly from the fifth vertebra. The upper tracing is the heart record; the middle is respiration. The large intervals on the time-signal record are ten seconds. *A*, the effect of sticking several times with a sharp needle. *B*, the needle was inserted gently just beneath the epicardium. *C*, weak faradic stimulation of the ventricle after all branches of the vagi were cut except the post-branchial ramus of the fourth branchial division on each side. The hypobranchial and other spinal nerves from the intact portion of the cord were also cut.

reflex movements in the anterior gill region, apparently through the more anterior cranial nerves. It therefore appears that the vagus innervation of the heart, both efferent and afferent, is by way of two pairs of branches, one pair from the post-branchial ramus of the

fourth branchial division, as in *Squalus*, and the other pair from the visceral branch of the vagus.

Cadiat (1879) showed that double vagotomy in *Scyllium* results in an increased rate of the heart. McWilliam (1885) and Kolff (1908) have found the same result in teleosts. The writer found a similar effect (Table I). In some instances cutting one vagus gave a considerable increase in rate which was further augmented when the remaining vagus was cut. The effect is most strikingly seen when the initial rate is low. Such figures have been taken to mean that the vagus normally exerts a tonic influence on the heart. In view of the great ease with which reflex inhibition of the heart occurs in elasmobranchs, as shown by Schoenlein and Willem (1894), Lyon (1926), and Lutz (1929), it is doubtful whether the result of vagotomy under the usual experimental conditions is an indication of the existence of normal vagal tone. In one case the initial rate, some time after exposing the heart, was 30 per minute. After cutting the previously prepared left vagus the rate was 34. Following an hour of experimentation, consisting of producing reflex inhibition by various means, the rate fell to 12. Then cutting the right vagus resulted in an increase to 30. Even gentle blowing on a wound is sufficient to produce a considerable reflex slowing of the heart.

TABLE I

The effect of vagotomy on the rate of the heart of Scyllium canicula, showing an apparent release from vagal tone.

Before vagotomy *Beats per minute*	After double vagotomy	Before vagotomy *Beats per minute*	After cutting one vagus	After cutting second vagus
30	44	16	33	42
24	40	36	36	38
22	36	26	32	36
18	32	32	32	40
20	34	24	24	38
36	44	39	42	44

Many attempts were made to obtain acceleration of the heart by stimulating the medulla or the cord with the vagi cut and with the gills perfused, but, confirming Bottazzi (1902), no acceleration occurred. The vagus was cut high, or in some cases the medulla itself was destroyed, in order not to interfere with any possible union of sympathetic fibers from the hypobranchial or other roots of spinal nerves with the vagus, although a sympathetic supply from these sources was

considered quite unlikely for morphological reasons. The cord was pithed posterior to the eleventh vertebra and a section three centimeters long was exposed anterior to this vertebra. In *Squalus* this section of the cord (fifth to twelfth vertebra) gives rise to fibers passing to the first sympathetic ganglion, according to Müller and Liljestrand (1918). Faradic stimulation of either the exposed cord or the anterior cut surface of the medulla or mid-brain did not produce acceleration of the heart. Neither did faradic stimulation of the first large sympathetic ganglion accelerate the heart. In this case any possible accelerator pathway from the ganglion to the heart by way of the cardiac ramus of the vagus was not disturbed.

Since there is some evidence that the vagus center normally has a tonic inhibitory effect on the heart, and since a period of acceleration is sometimes seen following a period of inhibition, it was considered that during activity of the cardio-inhibitory center there might be a lowering of tone in the accelerator center. After a period of increased activity of the vagus center its tone, through fatigue, might be lowered and this with the return of the accelerator center to its former condition might account for the acceleration sometimes seen. With the vagi cut and the vagus center active a lowering of accelerator tone would be expected to result in slowing of the heart. To test this a preparation with the medulla and cord to the twelfth vertebra intact was used with the gills perfused. Various means shown previously (Lutz, 1929) to produce marked reflex cardio-inhibition were used, such as faradic stimulation of the central end of the cut vagus or the cut lateral line nerve, vigorous mechanical stimulation of the nasal openings, or pinching the sides of the fish between heavy forceps. The records of the heart rate during such procedures did not show slowing, and consequently it is concluded that no lowering of tone in an accelerator center occurred. This can not be taken as complete proof, however, that no accelerator center exists, but since, in addition, no accelerator fibers have been demonstrated, it points strongly to its absence. Acceleration of the heart sometimes seen after a period of inhibition is probably due to fatigue of the inhibitory center.

I wish to thank Dr. Reinhard Dohrn and Dr. Enrico Sereni for the many courtesies extended at the Zoölogical Station, Naples, and Edna B. Lutz for technical assistance.

Summary

1. Two branches of the vagus go to the heart of *Scyllium canicula*, each carrying afferent and efferent fibers, one from the post-branchial

ramus of the fourth branchial division (fifth branchial nerve), and the other from the main visceral trunk.

2. No acceleration of the rate of the heart occurred on faradic stimulation of the medulla, spinal cord, or first large sympathetic ganglion with the vagi cut.

3. No slowing of the rate of the heart occurred, due to lowering of tone in an accelerator center, on reflex excitation of the cardio-inhibitory center with the vagi cut.

4. Both morphological and physiological evidence indicate the lack of accelerator fibers to the heart of the elasmobranch.

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THE VISCERAL AFFERENT PATHWAY IN THE ELASMOBRANCH, *SCYLLIUM CANICULA*

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With the elaborate morphological development of the autonomic nervous system in the higher vertebrates, the efferent innervation of the abdominal viscera has tended toward physiological differentiation. However, while the orthodox view holds that the cranial parasympathetic carries the excitatory fibers for the stomach and intestines, and the sympathetic contains the inhibitory fibers, Langley (1898) found inhibitory fibers to the stomach in the vagus of the rabbit, Morat (1893) found excitatory fibers to the stomach and intestine in the splanchnic of the dog, and Carlson, Boyd and Pearcy (1922) have found that both splanchnics and vagi of the cat carry both kinds of fibers to the stomach. Even in the elasmobranch fishes, where the autonomic nervous system is still morphologically simple, the two motor functions are not distinctly separated. Bottazzi (1901) and Müller and Liljestrand (1918) have reported motor activity of the stomach on stimulation of either the vagus or the first sympathetic ganglion, and the latter workers report also inhibitory bundles in the vagus.

On the afferent side of visceral innervation there is ample evidence in the higher vertebrates that there are fibers running from the abdominal viscera to the cord by way of the autonomic system (Head, 1893; Ranson and Billingsley, 1918). Stimulation of the stomach causes acceleration of the heart, according to Dmitrenko (Ranson, 1921), through afferent fibers mainly in the splanchnic nerve, but to some extent in the vagus. Brodie and Russell (1900) obtained slowing of the heart on stimulation of the central ends of the vagi to the stomach and concluded that afferent impulses are carried up the vagi as well as along the splanchnics in the cat.

In the elasmobranch fishes one might expect the vagi to take the larger part in carrying afferent fibers from the abdominal viscera although the writer found in the literature no reference to the vagus as an afferent pathway from these organs. Lyon (1926) noted cardio-inhibition on stimulating the stomach and other abdominal organs in

sand sharks (*Carcharias*), but reported no attempt to locate the afferent pathway. In a teleostean fish (eel) McWilliam (1885) could obtain no cardio-inhibition on stimulation of the abdominal organs, and while stimulation of the central end of the vagus on the oesophagus caused a marked cardio-inhibitory response, stimulation of either vagus after it had passed to the stomach had no effect. Mills (1886), however, working on the teleost, *Batrachus tau*, never failed to get reflex cardio-inhibition from the stomach and intestine, but he made no examination of the nervous pathways involved.

Materials and Method

The method of operating on *Scyllium canicula* out of water, and of recording the heart and respiratory rates has been described in a previous paper (Lutz, 1930). The spinal cord was transected at a desired level and pithed posteriorly. The gills were perfused through the mouth and respiration quickly became regular. The vagus and its branches were exposed through the anterior cardinal sinuses. The first large sympathetic ganglion and accessory ganglia were exposed through the posterior cardinal sinuses. Both operations were, of course, accompanied by unavoidable profuse bleeding, but since the writer found that the medullary centers of *Scyllium* would continue to be reflexly functional for about one hour after aortic transection, the bleeding did not interfere.

Results

Mechanical and faradic stimulation of the stomach, spiral valve, or mesentery caused cardiac and respiratory inhibition when the spinal cord was destroyed posteriorly from the first vertebra, but not when the vagi were cut either immediately posterior to the origin of the fourth branchial division (fifth branchial nerve) or just posterior to the origin of the cardiac rami from the visceral branches (Figs. 1 and 2, *A*, *B* and *C*). With the medulla, cord to the fourteenth vertebra, and cardiac vagi intact neither cardiac nor respiratory inhibition could be elicited on faradic stimulation of the first large sympathetic ganglion, but the same stimulus applied to the parietal peritoneum 3 mm. from the ganglion or to the adjacent oesophageal wall produced marked cardiac diastolic inhibition and sometimes respiratory inhibition as well (Fig. 2, *E* and *F*). Removal of the large sympathetic ganglion on each side and several accessory ganglia posterior to it or destruction of the spinal cord as high as the first vertebra failed to prevent elicitation of the inhibitory responses on stimulation of the stomach, spiral valve, or mesentery so long as the visceral

branches of the vagi remained intact. Faradic stimulation of the central end of either visceral branch of the vagus, cut at the stomach, also produced cardio-inhibition. Vigorous mechanical or faradic

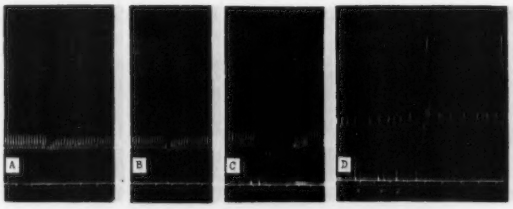

FIG. 1. Reflex cardiac and respiratory responses on stimulation of the abdominal viscera. Forebrain and cord from the third vertebra destroyed. Upper record, heart; middle, respiration. The large divisions on the time-signal record are ten seconds. *A*, spiral valve pinched with forceps. *B*, stomach pinched. *C*, gentle blowing on the viscera. *D*, the vagi have been cut below the cardiac rami. *x*, spiral valve pinched. *y*, stomach pinched. *z*, points of the forceps inserted in the nasal openings.

stimulation of the liver and the testis failed to evoke either cardio-inhibition or respiratory slowing even with the entire cord intact. Stimulation of the kidney, epididymis, ovary, and uterus were effec-

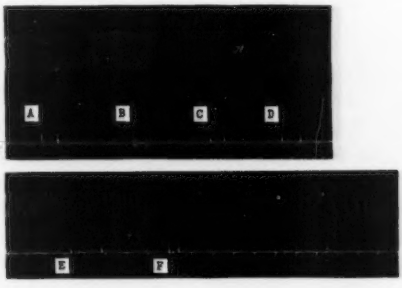

FIG. 2. Reflex cardiac inhibition. Forebrain and cord from the thirteenth vertebra destroyed. Vagi cut below the cardiac rami. *A*, stomach pinched with forceps. *B*, faradic stimulation of the stomach. *C*, spiral valve pinched. *D*, forceps inserted in the nasal openings. *E*, faradic stimulation of the first large sympathetic ganglion. *F*, faradic stimulation of the peritoneum 5 mm. from the ganglion.

tive in producing both reflexes when the cord was not destroyed (Fig. 3). Stimulation of the kidney was without effect with the cord pithed posterior to the third vertebra. No attempt was made to locate the portion of the cord receiving the afferent fibers, and no examination

of the relation of the vagus to the kidney, epididymis, ovary, and uterus was made.

The results presented above indicate that in the elasmobranch, *Scyllium canicula*, the vagus carries afferent fibers from the stomach, spiral valve, and mesentery. In view of the fact that sensory stimu-

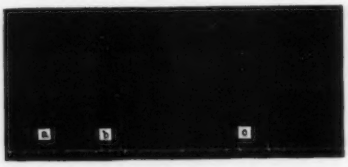

FIG. 3. Reflex cardiac inhibition. Entire cord intact. *a*, uterus pinched with forceps. *b* and *c*, ovary pinched. *x*, viscera handled. *y*, ovary handled.

lation at a great variety of points produces reflex cardiac and respiratory inhibition in *Scyllium* (Lutz, 1929), the failure of stimulation of the first large splanchnic ganglion to elicit these responses may be taken to indicate a lack of afferent fibers through this ganglion. The liver and testis also have no afferent fibers, stimulation of which gives either cardiac or respiratory response. Lyon (1926) likewise obtained no cardio-inhibition on stimulation of the liver in sand sharks (*Carcharias*).

SUMMARY

1. The vagus in the elasmobranch, *Scyllium canicula*, is an afferent pathway from the stomach, spiral valve, and mesentery.

2. There are no afferent fibers passing through the first large sympathetic ganglion or from the liver and testis, stimulation of which produces either cardiac or respiratory inhibition.

3. Removal of the sympathetic ganglia or destruction of the spinal cord as high as the first vertebra fails to prevent elicitation of the inhibitory reflexes provided the visceral branches of the vagi remain intact.

4. Faradic stimulation of the central end of a visceral branch of the vagus at the stomach produces cardiac and respiratory inhibition.

I wish to thank Dr. Reinhard Dohrn and Dr. Enrico Sereni for the many courtesies extended at the Zoölogical Station, Naples, and Edna B. Lutz for technical assistance.

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NOTES ON A FRESH-WATER MEDUSA FOUND IN STALLWORTH LAKE, TUSCALOOSA, ALABAMA

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During a series of collecting trips, fresh-water medusæ were found in Stallworth Lake near Tuscaloosa, Alabama (White, 1929). They were first observed on September 14, 1928. I visited the lake at least twice a week and never failed to find medusæ until the ninth of October. They did not reappear after this date, although I continued to visit the lake at regular intervals. The approach of cold weather does not seem to have caused their disappearance, as has been suggested in other cases, since warm weather persisted for several weeks after they were last seen. Specimens, which I had placed in large jars of pond water in the laboratory, lived for about two weeks longer, gradually wasting away in size and finally dying. The water contained small Crustacea, so it is hardly possible that lack of food caused their death.

The medusæ were sensitive to changes in intensity of light and to disturbances in the water. In general they were more abundant at the surface on bright days than on cloudy ones. Although I could not bring the medusæ to the surface of the lake at night by shining a flashlight on the water, they became very active when the lights were suddenly turned on in a dark laboratory. I have brought them to the surface by violently thrashing the water at a considerable depth with a long board or iron pipe. They also became very active when the water of the aquarium was stirred.

No hydroid stages were found although repeated searches were made for them from time to time during the fall of 1928 and the spring of 1929. Quantities of scrapings from the piles of the pier and the surfaces of submerged boards and rocks were examined. The hydroid stage was probably present, however, and I believe that if a more extensive search had been made, polyps would have been found in considerable numbers, since the medusæ were so abundant. Perhaps the hydroids were confined to a few localized areas, which would account for my not finding them.

The medusæ have not been observed in the lake since October, 1928. I examined the lake at frequent intervals during the entire summer of 1929 but did not see a single medusa.

The Lake

Stallworth Lake is situated on a terrace midway between the business district of Tuscaloosa, Alabama, and the Warrior River. It was formed in 1918 by damming up four acres of marshy land and is supplied by springs which keep a small stream running from the lake. This streamlet flows into a small pond which finally empties into the river. The depth of the lake varies from a few inches to about twenty feet. Along the edges the bottom is sandy, but in the deepest parts it is covered with mud. There is an abundance of plant life in the lake. Fresh-water algæ give the water a greenish color. Several species of the higher plants are also present. Willows line the dam on one side of the lake. The fauna of the lake is abundant and varied. Numerous species of Protozoa and members of the other invertebrate phyla were constantly encountered during the search which was undertaken for the hydroid. Fish have been introduced from hatcheries in Mississippi and the Warrior River. A large number of turtles are found in the lake, no doubt coming in from the river. Mussels of the genus *Lampsilis* abound in the lake as do snails of the genus *Physa*. In the spring and early summer the lake is used as a swimming pool. Later it is little frequented due to the rather slow change of water. There is a pier, extending out over the water from one shore, which connects the boat house with a small island. A part of this pier is covered by a roof. Most of the specimens were collected from the water on the sides of this pier.

Although the Warrior River is subject to sudden rises which spread it over the surrounding territory, the level of the river has never reached that of the lake. The lake has never been drained, but every year the water is lowered about two feet to facilitate cleaning and repairing. The pH of the water at the time the medusæ were found was 7.2. As for the temperature of the lake, it never freezes, and in the summer the surface may reach a temperature of eighty-five degrees Fahrenheit, due to the very slow turnover of the water.

Historical Survey of the Fresh-water Medusæ

Although marine medusæ have been known for centuries (Aristotle mentions several species), a fresh-water medusa was not reported until 1880. In June of that year Mr. Sowerby, secretary of the Royal Botanical Society, found medusæ in the Society's *Victoria regia* tank in Regent's Park, London. He gave specimens to Lankester and Allman who studied and described them. Allman proposed the name *Limnocodium victoria* for the new form, while Lankester called it *Craspedacusta sowerbii*, in allusion to the relation of the otocysts to the velum. For

a number of years the medusa was known as *Limnocodium sowerbii*, which evidently violates the accepted rules of nomenclature. An attempt was made to have this name validated, but the petition was denied by the International Commission on Zoölogical Nomenclature (Mayer, 1910). Lankester's name, *Craspedacusta sowerbii*, published on June 17, 1880, has priority, and is clearly the correct one. I call attention to this point since the name *Limnocodium* has appeared in the literature within the last two years. *Craspedacusta sowerbii* appeared in the lily-tank of Regent's Park for a number of years, finally disappearing in 1893. Romanes (1880) reported some interesting experiments on the physiology of this fresh-water medusa. Bourne (1884) first described the hydroid stage. Fowler (1890) described medusoid bud formation and gave a complete bibliography of the literature up to that time. Günther (1894) worked out the histology of the medusa stage of this form.

In the last half-century fresh-water medusæ have been reported many times from widely separated localities. Edward Potts (1897) reported the first fresh-water species from America. He had already found the hydroid stage of this form (1885) and had called it *Microhydra ryderi*. Ryder (1885), believing that *Microhydra ryderi* was probably the hydroid stage of a medusa and that this medusa would prove to be generically different from *Craspedacusta*, because of certain differences between *Microhydra ryderi* and the hydroid stage of *C. sowerbii*, proposed the generic name Pottsia, should the medusa stage be found. Why he should have favored separate names for the hydroid and the medusa is not clear. At any rate the name was not valid and was never used. Payne (1924) has demonstrated that *Microhydra ryderi* is a species of the genus *Craspedacusta*, and has designated it *Craspedacusta ryderi*, abolishing the genus *Microhydra*. He reported the complete life cycle of this species (1926).

Although the evidence is not conclusive and only a study of the development could permit a final decision, presumably, the medusæ found by Hargitt (1907), Coker (Payne, 1924), and Garman (1916), and assigned to the European species *Craspedacusta sowerbii*, are specifically identical with the forms found in Boss Lake, Indiana (Payne, 1924) and in Stallworth Lake, Alabama, and should be referred to the American species, *Craspedacusta ryderi*.

Roch (1924) found fresh-water medusæ in a mill stream near Berlin. The largest specimens measured 0.68 millimeter in diameter. They differed from the young medusa of *Craspedacusta ryderi* (then called *Microhydra ryderi*) in having sixteen tentacles which were not of uniform length and which did not appear simultaneously in the course

of the development of the medusa. He also stated that the great geographical separation of this form from *Microhydra ryderi* spoke little for their identity. To this form he gave the name *Microhydra germanica*. It is, no doubt, a member of the genus *Craspedacusta*, and whether it is a different species from *Craspedacusta sowerbii*, the European form, can only be determined by a study of the hydroid, budding, and medusoid bud formation. If, indeed, it is a different species, the correct name would be *Craspedacusta germanica*.

Other species of fresh-water medusæ which have been reported up to the present time are *Limnocnida tanganjicæ* (Günther, 1893) from Africa, *Craspedacusta kawaii* (Oka, 1908) from China, and *Limnocnida indica* (Annandale, 1912) from India. Payne (1924, 1926) gives an extensive bibliography covering fresh-water medusæ to that date. Other papers not listed are cited here. These include a report by Goette (1908), who found them in Strassburg. Pelosse (1919) found them in a park in Lyon. Backhoff (1924) reported them from Stettin. Flower and Lockyer (1928) reported the reappearance of *Craspedacusta sowerbii* in the Royal Botanic Society's garden in Regent's Park, London, where fresh-water medusæ were first reported in 1880. Rupert Vallentin (1930) found medusæ in the Exeter ship canal in July of 1928 and 1929.

The Adult Medusa

Payne (1924) has given an excellent description of the medusa of *Craspedacusta ryderi*. Since the medusæ of Stallworth Lake conform very closely to his description, I shall give here merely a few details wherein they differ from those found in Boss Lake. Payne found that the sexes were so alike that the only way to tell them apart was by an examination of the gonads. I found only males, although a number of the medusæ were examined by smear and sectioning methods. I am inclined to think that if females were present, at all, they occurred in very small numbers. As I did not arrive in Tuscaloosa until early fall, all the medusæ were fairly large—none measuring less than fourteen millimeters in diameter. The medusæ are, evidently, slightly larger than those found by Payne, the largest specimens measuring about twenty millimeters in diameter, when in the relaxed condition. There is a correspondingly greater number of tentacles—over four hundred were counted on each of several individuals. Although the tentacles of *Craspedacusta* have, heretofore, been placed in three size groups, they seem to fall into four fairly distinct sets. Allman (1880) shows this clearly in his drawing, but uses only three groups in his description. First, there are the perradials, four large tentacles lying

at the terminations of the radial canals. Those of the next group are almost as large and lie between the perradials. There are about twenty-five of these. The third set is considerably smaller than the first two. There are about thirty-five in this group, which are scattered among the larger tentacles. The smallest tentacles form a hair-like fringe around the periphery of the umbrella. They are so numerous that they are difficult to count. Three hundred and thirty-eight were counted on one individual. Payne (1924) found sixty-nine lithocysts in a specimen five millimeters in diameter. I have counted over two hundred in several mature specimens.

These differences may have been caused by developmental differences or by dissimiliar environmental conditions. In other respects the medusæ from the two localities are quite similar. The study of the hydroid, if it is found, and the developmental processes may, possibly, bring out specific differences between the Alabama and Indiana forms, but at the present time I am inclined to think that the medusæ are specifically identical and have tentatively assigned the form from Stallworth Lake to the species *Craspedacusta ryderi*.

Spermatogenesis

When the medusæ were first observed specimens were taken to the laboratory. The gonads of a number of these were excised and fixed in Bouin's fluid. Sections of these gonads were cut at five microns and stained with iron hæmatoxylin. Others were mordanted in Flemming's fixative and stained with Flemming's triple stain. These sections demonstrated that all of the medusæ examined were males. In spite of the fact that all of the medusæ were fully grown, various stages of spermatogenesis were observed in each section. Since no detailed study of spermatogenesis in a fresh-water medusa has been reported, certain observations will be given here.

From the subumbrella side of each radial canal there projects a long sac-like pouch, lined by a layer of entodermal epithelium, a single cell in thickness, which is continuous with that of the radial canal and which forms the cavity of the gonad. The mesogloea is much reduced in the gonad, forming only a very thin layer at the base of the entoderm cells. The rest of the wall of the gonad is thick and composed of germ cells and developing sperm, with a thin superficial covering of ectodermal tissue. The entoderm cells are columnar in shape with large irregular vacuoles in their cytoplasm. The nuclei are found in the end of the cells distal to the cavity of the gonad. They contain large centrally placed nucleoli. Adjacent to this entodermal layer are the spermatogonia. Since only mature gonads were secured, no in-

formation can be given concerning the origin of the germ cells. Günther (1894) considered them to be of ectodermal origin. He distinguished sperm mother cells (spermatogonia), daughter spermatoblasts (secondary spermatocytes), spermatids, and spermatozoa, but found them to be too small for any accurate observations. The spermatogonia lie nearest the entoderm, while the later stages occur further toward the outside of the gonad. The spermatozoa are found just under the layer of ectoderm. They are, presumably, shed by rupturing this thin superficial layer.

The spermatogonia form a closely packed layer nearest the entoderm. They are irregular cells, having a diameter of about six microns. The nuclei are large, filling a considerable part of the total volume of the cell. They have large deeply staining nucleoli, which are usually centrally placed. The nucleolus is generally single, although double ones occur. The nuclear membrane is delicate but can be readily seen under 1.5 oil immersion lens. The scarcity of mitotic figures among the spermatogonia indicates that division of these stages had stopped in the gonads under observation. The condition of the chromatin, packed into a single large nucleolus, seems to be the typical resting stage of the spermatogonia. Figure 1 shows a resting spermatogonium.

The first indication of the change from spermatogonia to primary spermatocytes is a fragmenting of the large nucleolus. It may break into several pieces which gradually diminish in size. In a number of cases, however, the nucleolus breaks into two portions which move apart within the nucleus before fragmentation begins. When this process does start, one of the pieces disappears before the other, leaving for a time a large dark staining body at one side of the nucleus and finely granular chromatin at the other. As fragmentation of the nucleolus continues the chromatin becomes finely granular and is practically unstainable with iron hæmatoxylin. It stains with safranin, however, in this condition. In cells adjacent to those in the granular stage, leptotene threads begin to appear. The chromatin at this stage forms knots which are suspended just under the nuclear membrane. The nucleus has attained its maximum size at this time. From this irregular network heavy threads develop. There is evidently an increase in the total quantity of chromatin, certainly in the stainable part, since the amount in the heavy syndesis mass shown in Fig. 6 is much greater than the delicate mesh shown in Fig. 5. The nucleus is not as large at this stage as it was in the preceding one.

The next change which can be observed is the disappearance of the nuclear membrane and a definite flattening of the mass to form

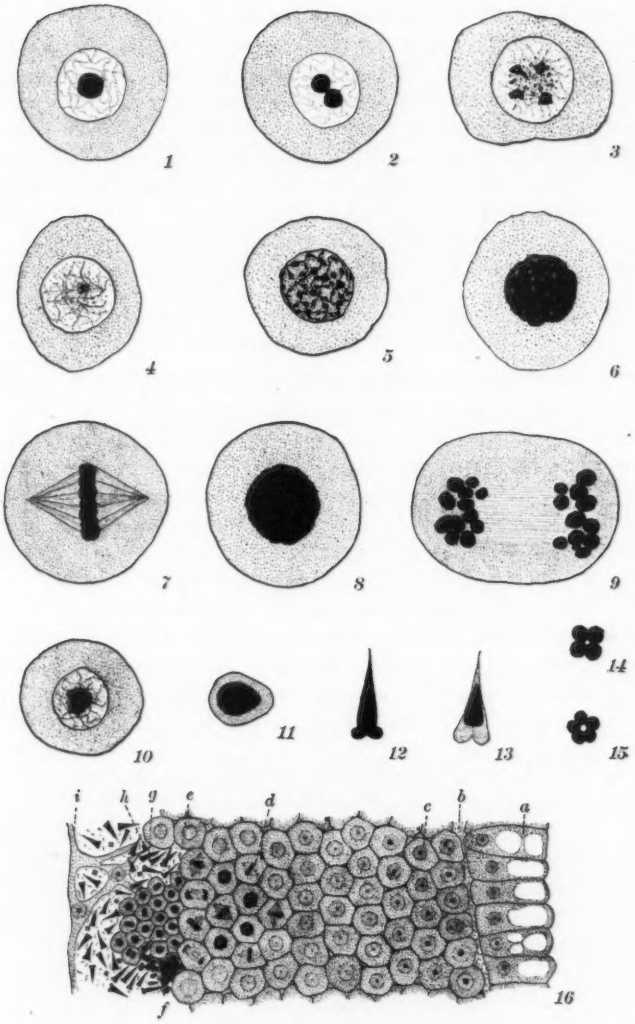
an equatorial plate. There are no central bodies visible, but spindle fibres are present. Because of the compactness of this equatorial plate, individual chromosomes are not readily distinguished at this stage. The plate soon splits and the chromosomes start a typical migration towards the poles of the cell. Only after this migration is nearly finished can individual chromosomes be observed. In this late anaphase twelve chromosomes have been counted in each end of a number of cells. The chromosomes are very small and are difficult to count, but there seem to be twelve at each pole at the late anaphase of the primary spermatocytes. Figure 9 is a diagrammatic sketch of the general arrangement of the chromosomes at this stage. They are not as clearly distinguishable as indicated in this figure.

The secondary spermatocytes are readily distinguished from the primary ones by their smaller size. At first the chromatin is in the form of a coarse densely packed mesh. It soon forms a compact knot which gradually changes to the equatorial plate of the spindle. The meiotic process continues without a pause, forming two daughter spermatids. The details of this division are difficult to observe because of the small size of the cells and the masking, caused by the compactness of the chromatin. The chromatin masses can be resolved into individual chromosomes only with great difficulty in the secondary spermatocytes. Figure 10 shows a secondary spermatocyte.

EXPLANATION OF PLATE

The figures of this plate, unless otherwise indicated, represent a magnification of 3500 diameters as they are reproduced. Figure 10 is drawn at a magnification of 4000 diameters. Figure 16 represents a magnification of 700 diameters. The figures were drawn with the aid of a 1.5 oil immersion (Spencer) and a 10 × ocular (Spencer). The drawings are all from sections.

FIG. 1. Resting spermatogonium showing large chromatin-nucleolus.
FIG. 2. Spermatogonium with double nucleolus.
FIG. 3. Primary spermatocyte showing fragmentation of the nucleolus.
FIG. 4. Primary spermatocyte with chromatin in finely granular state.
FIG. 5. Later stage of development.
FIG. 6. Pachytene stage.
FIG. 7. Metaphase of primary spermatocyte.
FIG. 8. Polar view of primary spermatocyte showing equatorial plate in which individual chromosomes are not readily distinguished.
FIG. 9. Diagrammatic sketch of late anaphase showing general arrangement of chromosomes. They are not as distinct as represented in this figure.
FIG. 10. Secondary spermatocyte with massed chromatin.
FIG. 11. Spermatid which has begun to elongate.
FIG. 12. Sperm stained with iron hæmatoxylin.
FIG. 13. Sperm stained by Flemming's triple method.
FIGS. 14 and 15. Axial views of the sperm in the region of the knobs.
FIG. 16. Section through the wall of the gonad. *a*. entoderm; *b*. mesogloea; *c*. spermatogonia; *d*. primary spermatocytes; *e*. secondary spermatocytes; *f*. spermatids; *g*. sperm; *h*. axial view of sperm; *i*. ectoderm.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The spermatids are at first small and round. They gradually draw out at one side. This continues until there is a distinct process projecting from one side, at the base of which are four or five balls or knobs. There are generally four knobs, but five are sometimes present. These knobs stain very intensely with iron hæmatoxylin, but stain only very slightly with safranin. The process takes iron hæmatoxylin stain, but not so intensely as the knobs, and is more readily destained. Figure 12 shows a sperm stained with iron hæmatoxylin. Figures 14 and 15 are axial views of the sperm in the region of the knobs, similarly stained. Figure 13 is a drawing of a sperm stained by Flemming's triple method. There is no indication of a flagellum in fixed material, but Vaney and Conte (1901) observed live sperm and reported the presence of a flagellum at the base of the knobs. If this observation is correct, the long pointed part is the anterior end of the sperm.

Summary

1. Fresh-water medusæ were observed in Stallworth Lake, an artificial body of water near Tuscaloosa, Alabama. They were first seen by the writer on September 14, 1928. They disappeared on October ninth of the same year and have not been observed since.

2. The hydroid stage was not found.

3. A description of the lake where the medusæ were found is given.

4. A historical survey of the fresh-water medusæ is made.

5. The medusæ agree very closely with *Craspedacusta ryderi* as described by Payne (1924). There are certain differences which may be developmental or due to dissimilar environmental conditions. The medusæ are assigned to the species *C. ryderi*.

6. The tentacles are grouped into four, instead of the three previously used groups.

7. Sections of the gonads demonstrated that all the medusæ examined were males.

8. Observations on the spermatogenesis of *C. ryderi* are included.

9. Since all gonads obtained were from adult medusæ, no information concerning the origin of the germ cells can be given.

10. The primary spermatocytes are only very slightly larger than the spermatogonia.

11. There is a meiotic stage in which the chromatin is stained only very poorly with iron hæmatoxylin.

12. There seem to be twelve chromosomes at each pole of the late anaphases of the primary spermatocytes.

13. Some portions of the sperm are stained more intensely by

Flemming's triple method and others by iron hæmatoxylin. Thus the two methods give different appearances to the sperm.

14. Tails are not visible on sperm in fixed and stained preparations.

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THE LONGEVITY OF UNFERTILIZED GAMETES

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INTRODUCTION

Observations which have been reported on the aging process of unfertilized gametes and upon chemical phenomena associated with fertilization make it desirable that these processes be studied in several widely separated species. It is important to know whether the described phenomena are of wide or of limited occurrence.

The conclusion of Goldforb that the eggs of different individuals of the same species are very diverse, and that failure to take this into account may vitiate experimental studies upon these eggs is a matter deserving consideration, especially by those whose work involves the use of eggs and developing embryos.

The suggestion made by F. R. Lillie that the gametes, both eggs and spermatozoa, contain substances which are essential to fertilization deserves critical attention. It is assumed that these particular chemical substances are gradually lost by the gametes to the water, so that, if a spermatozoön does not promptly meet with an egg after being shed, it loses its ability to fertilize an egg or to initiate the fertilization reaction. This theory accounts for the well-known phenomenon that spermatozoa in dilute suspensions lose their power to fertilize more quickly than is the case with concentrated suspensions. This phenomenon, however, may be accounted for on the theory that the more concentrated suspensions contain more of the longer-lived spermatozoa and therefore appear to retain vitality longer than dilute suspensions. Cohn (1918) explains it by saying that spermatozoa are endowed with a limited amount of energy. When this is used up their movements cease. In concentrated suspensions, because of increased hydrogen ion concentration brought about by the production of carbon dioxide, the activity of the spermatozoa is lessened, and they live for a longer time.

Aside from these more fundamental problems, we have had in mind to determine how long eggs may retain vitality and be capable of fertilization and normal development in case they fail to meet a spermatozoön. In a former paper the senior author published results of experiments upon the eggs and sperm of the lamellibranch, *Cumingia*

tellinoides, in which it was shown that spermatozoa may, even in dilute suspensions, retain their ability to fertilize eggs for several hours and that so long as they are able to swim they retain this ability.[1] Experiments on the gametes of the annelid, *Hydroides hexagonis*, made during the summer seasons of 1928 and 1929, confirm the interpretation given in the paper on *Cumingia*.

The Egg of Hydroides

The eggs of *Hydroides hexagonis* are comparatively small, measuring 0.057 to 0.063 mm. They are protected by a heavy vitelline membrane, and the germinal vesicle is normally intact until the spermatozoön enters the egg.

They are readily obtained during the summer at any time between June 20 and September 20. However, throughout the early part of the breeding season, the mature are mixed with the immature eggs to such an extent as to render their study difficult. Late in July and during the first half of August the eggs of this species are in their best condition and almost free from immature eggs. Our studies were therefore made chiefly during the height of the breeding period, thus avoiding also the latter end of the season, when the eggs are found to be inferior in vitality and erratic in their behavior. It has become evident that the last eggs produced are in a weakened condition, presumably from too long storage in the cœlomic cavities. Goldforb believes that long storage is the chief cause of the variation in longevity shown by the eggs of *Arbacia*. We believe that this is the cause of the inferiority of the eggs of *Hydroides* which was noticed toward the end of the breeding season.

Longevity of Eggs

In each of our experiments the eggs of eight to ten females, spawned during the same hour, were kept without fertilization in as many Stender dishes. The water was changed frequently to provide aëration and reduce infection. At regular intervals a few eggs from each lot were examined microscopically and note was made of physical changes which indicate deterioration or death by disintegration. At the same intervals a few eggs of each lot were inseminated with fresh sperm to ascertain the rate of deterioration as indicated by the percentage that failed to fertilize and cleave. Numerous experiments of this type were carried out, the results of which show that the poorest lots of eggs disintegrate within six or eight hours, while the longest-lived eggs survive for thirty-two hours. The variation in longevity

[1] "Vitality of the Gametes of *Cumingia tellinoides*," *Biol. Bull.* 1928.

within a single lot of eggs is greater than in the cases of eggs of other species studied to date. The average longevity of the unfertilized eggs of this species appears to be between eighteen and twenty-four hours. It was shown that the most vigorous lots of eggs that were fertilized after they were from eighteen to twenty-four hours old, gave rise to abundant normal trochophore larvæ. The oldest eggs that gave rise to normal larvæ were twenty-six hours old. Many eggs even from the first die in cleavage stages, and the percentage which fail to become normal larvæ increases with age. Table I shows the variation observed in the longevity of unfertilized eggs. It includes selected examples to show the whole range of variation which has been found by two years study of this species.

The shortest-lived lots are given at the top of the table, and it is shown that all of the eggs of females I and II died in eight or nine hours, while a small percentage of the longest-lived eggs of females X and XII were capable of fertilization after thirty-two hours. The average longevity appears to be about eighteen to twenty-four hours.

The longevity of the eggs was tested also by the method of disintegration, a living egg being readily distinguished from one that is dead. When an egg dies, the vitelline membrane remains intact, but the protoplasmic contents either become compact and lobulated like a raspberry or a golf ball, or it disintegrates and the egg becomes swollen.

TABLE I

The comparative longevity of the unfertilized eggs of various females of hydroides hexagonis as indicated by the decreasing percentage of cleavage with age. A few eggs of each lot were fertilized at intervals as shown in the several columns of the table.

Female No.	Percentage of Cleavage with Aging			
	after 1 hour	after 5 hours	after 8 hours	after 10 hours
	per cent	*per cent*	*per cent*	*per cent*
I	80	27	4	0
II	78	60	2	0
III	77	0	65	0
IV	98	0	90	0
V	90	0	90	0
VI	98	0	92	0
VII	92	0	0	0
VIII	99	90	0	0
IX	96	91	0	0
X	92	78	67	0
XI	95	91	0	0
XII	93	0	0	0
XIII	97	96	0	0

TABLE I—*Continued*

Female No.	after 18 hours	after 24 hours	after 28 hours	after 32 hours
	per cent	*per cent*	*per cent*	*per cent*
I	0	0	0	0
II	0	0	0	0
III	3	0	0	0
IV	49	26	0	0
V	26	12	0	0
VI	38	10	0	0
VII	40	16	0	0
VIII	52	6	0	0
IX	22	14	2	0
X	0	48	17	2
XI	0	28	6	0
XII	50	45	12	5
XIII	0	8	0	0

By making counts of the eggs at intervals and applying this test, the variation in the longevity of the eggs of various females was determined.

Table II gives the longevity of the eggs of seven females as tested by the method of disintegration. It shows that the average longevity lies between eighteen and twenty-five hours.

TABLE II

The Longevity of Unfertilized Eggs of Hydroides hexagonis as Tested by the Method of Disintegration

Female No.	Percentage of Disintegration with Aging				
	after 1 hour	after 18 hours	after 25 hours	after 30 hours	after 32 hours
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
I	0	65	93	100	0
II	8	84	90	100	0
III	2	25	40	75	90
IV	0	30	48	85	95
V	2	75	88	100	0
VI	10	100	—	—	—
VII	20	100	—	—	—

THE LONGEVITY OF SPERMATOZOA

The longevity of the spermatozoa of *Hydroides* was studied in dilutions of 1/1000, 1/50,000, 1/100,000, 1/200,000, 1/400,000, 1/800,000, and 1/1,000,000. One drop of dry sperm added to 50 cc. of sea water was taken as a standard sperm suspension of 1/1000. The weaker

sperm suspensions were made from this standard suspension by appropriate dilutions.[2]

The questions sought to be answered were three: First. How long will spermatozoa live in sea water under natural conditions and retain their fertilizing potency? Second. Does the spermatozoön contain a soluble chemical substance essential to fertilization, and is this substance lost promptly by the spermatozoön to the sea water, thus rendering it incapable of fertilizing an egg? Third. How great must be the dilution of spermatozoa before capability to fertilize one hundred per cent of the eggs that are added to it is lost? (Is one sperm enough to fertilize an egg?)

TABLE III

Longevity of Spermatozoa of Hydroides, Aug. 16, 1929

Percentage Suspension	Percentage of Cleavage Obtained from Aging Sperm of Various Dilutions			
	after 1 hour	after 3 hours	after 5½ hours	after 8 hours
	per cent	*per cent*	*per cent*	
1/1,000 or 0.1 per cent	98	98	90	..
1/50,000 or 0.002 per cent	98	98	18	..
1/100,000 or 0.001 per cent	98	85	11	..
1/200,000 or 0.0005 per cent	98	83	17	..

Although a complete answer cannot be given to any of these questions, the data assembled throw light upon all. Tables III and IV show that all sperm dilutions from 1/1000 to 1/1,000,000 give practically 100 per cent fertilization shortly after the dilutions are made and that little deterioration has occurred after three hours. However, it is apparent after three hours that some of the spermatozoa are dying. After five hours the 1/1000 suspension still gives approximately ninety per cent cleavage, while cleavage in the weaker suspensions has fallen to twenty per cent or less. Other experiments show that not more than five to eight per cent cleavage may be expected from suspensions eight hours old. (Table IV) Nearly all of the spermatozoa are dead in dilute suspensions after seven or eight hours. Eight hours may, therefore, be taken as the approximate limit of the life of the spermatozoa of *Hydroides* in dilutions that may be comparable to conditions found in nature.

[2] The spermatozoa of *Hydroides* may be obtained dry by placing the worm upon a dry glass plate as soon as it has been removed from its calcareous tube. The sperm is promptly expelled from the nephridiopores all along the sides of the body. The pipette used in making dilutions gave twenty drops per cc. One drop of dry sperm in 50 cc. of water therefore gives a 1/1000 or 0.1 per cent. suspension. Ten drops of this standard sperm suspension in 50 cc. of water gives a 1/100,000 or 0.001 per cent sperm suspension, etc. One drop of the standard sperm suspension in 50 cc. of water gives a 1/1,000,000 or 0.0001 per cent sperm suspension.

If the spermatozoa of *Hydroides* contain a substance essential to fertilization, it is lost to the sea water only very slowly, because the spermatozoön retains its ability to fertilize an egg from three to eight hours and probably as long as it is able to swim.

It will be noted from Table IV that suspensions of 1/1,000,000 or 0.0001 per cent are able for a short time to fertilize almost 100 per cent of the eggs that are added to it. The potency of this extreme dilution is a partial answer to Glasser's question: "Is one spermatozoön capable of fertilizing an egg?"

TABLE IV

Longevity of spermatozoa of hydroides, Aug. 18, 1929. This table gives the longevity of spermatozoa from one male in various dilutions, including the extreme dilution of 1/1,000,000 or 0.0001 per cent.

Percentage Suspension	Percentage of Cleavage Obtained from Aging Sperm of Various Dilutions		
	after 1 hour	after 5 hours	after 8 hours
	per cent	*per cent*	*per cent*
1/1,000 or 0.1 per cent	94	85	5
1/50,000 or 0.002 per cent	93	84	1
1/100,000 or 0.001 per cent	93	83	4
1/200,000 or 0.0005 per cent	92	28	2
1/400,000 or 0.00025 per cent	93	26	1
1/800,000 or 0.000125 per cent	91	15	0
1/1,000,000 or 0.0001 per cent	92	8	1

The spermatozoa of *Hydroides* are unusual in one respect. When first expelled into sea water they are inactive and remain relatively so for almost an hour. Due probably to the stimulus of sea water they gradually become active and in the course of about half an hour are vigorous in their movements, but never so active as the spermatozoa of *Cumingia*, *Arbacia* or other species studied. It was anticipated that, because of its comparative lack of vigorous movement, this spermatozoön might survive for a longer period than other sperm, but this does not appear to be the case.

SUMMARY AND CONCLUSIONS

By way of summary, it may be said that unfertilized eggs of *Hydroides* which fail to meet a spermatozoön live and retain ability to be fertilized and develop into normal trochophores from eighteen to twenty-four hours. The extreme variation in the longevity of *Hydroides* eggs is six to thirty-two hours. Some lots of eggs at spawning are in poor physiological condition and one hundred per cent

of these die within eight hours. The best lots of eggs, on the other hand, show little deterioration under fifteen hours and a large percentage (40 to 50 per cent) of these are capable of normal development after twenty-four hours.

Spermatozoa, when expelled into sea water or mixed in dilute suspensions comparable to that obtaining in nature, live from three to seven hours and retain ability during that time to fertilize eggs.

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THE GOLGI APPARATUS OF AMOEBA PROTEUS PALLAS

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INTRODUCTION

The study of the Golgi apparatus and its identification in the Protozoa has been rendered almost impossible because the highly specialized Golgi techniques are not very specific, and they are very capricious even in the hands of our best technicians. Bowen (1928) has pointed out the fact that isolated gland cells often fail to respond to impregnation methods, and he suggests that similar trouble may be expected in protozoan techniques. Also the identification of the Golgi apparatus is impeded because just what may or may not be Golg. material has not been agreed upon by many of the investigatorsi Hirschler (1914) found spheres, crescents, and rings in *Monocystis agilis* and *Gregarina polymorpha*. King and Gatenby (1923) described similar crescents, and bead-like structures in *Adelia*. They believe these structures to be comparable to the dictyosomes of metazoan cells. Nassonov (1924, 1925) believes that the contractile vacuole is the homologue of the Golgi apparatus of metazoan cells. He found that the contractile vacuoles of *Paramecium*, *Lionotus*, *Chilodon*, and *Dogielella* stained by the Kolachev method of procedure. Duboscq and Grassé (1924–1927) believe that in *Holomastigotes*, *Pyrsonympha* and other related flagellates the parabasal bodies are the homologues of the Golgi apparatus. Grassé (1926) stated that since the euglenoid flagellates have no parabasal bodies, the stigma or eye-spot is a homologue of the Golgi apparatus. Causey (1925) finds a network in *Endamœba gingivalis;* this structure is similar to the Golgi network in metazoan tissues. Hirschler (1927) found rings, spheres, and crescents in *Endamœba blattæ* and he believes that these are the Golgi material. Joyet-Lavergne (1926) used neutral red as a vital stain on gregarines and found that these crescents and rings stained; therefore, he believes the structures to be Golgi elements. Hall (1930) finds in *Chilomonas paramecium* small granules and large vacuoles, and he believes that these bodies are comparable to the Golgi apparatus.

There is a great divergence of opinion as to just what may be or may not be Golgi material in the Protozoa. The structure and function of the Golgi apparatus are subjects of controversy. It is the aim

of the writer to describe and discuss the Golgi apparatus of *Amœba proteus* Pallas.

This work was done in the Zoölogical Laboratory of Johns Hopkins University and the writer wishes to take this occasion to thank Dr. Samuel O. Mast for numerous kindly favors and helpful coöperation.

Material and Technique

Amœba proteus was cultured on a cracked wheat infusion which was inoculated with *Chilomonas paramecium* and *Zoöchlorella*.

For fixation the centrifuge method of procedure was used. The Kolachev method as modified by Nassonov (1925) and Bowen's modification of the Mann-Kopsch procedure were used to good advantage. The Mann-Kopsch method gave the best results. Also Bowen's acid fuchsin-thionin-aurantia was slightly modified and used after a fixation in Champy's solution.

(1) Fix in Champy's solution 12 hours.
(2) Wash in distilled water.
(3) Pass the slides through graded alcohols to 70 per cent.
(4) Stain 10 minutes in acid fuchsin.
(5) Stain 15 seconds in very dilute thionin.
(6) Stain 5 seconds in dilute aurantia.

Dehydrate and mount in balsam.

Result: Golgi bodies are light blue with dark blue rims, the cytoplasm is pink, the nucleus is orange or vermillion, and the contractile vacuole and the granules around it are red.

The Golgi Apparatus of Amoeba

The Mann-Kopsch method of procedure brings out two types of granules in *Amœba proteus* which reduce osmic acid. One of these is a black granule and the other is a spherule with a black rim (Fig. 2, Plate). The black granules are similar to those described by Hall (1930) as occurring in *Chilomonas paramecium*. When the spherules are viewed at a central focus, they appear as rings, or crescents (Figs. 1–2, Plate). These bodies have clear centers. Both types are distributed at random throughout the endoplasm of *Amœba*, and often they have a tendency to flow out into the pseudopodia (Fig. 2, Plate). In no cases have they been observed to be associated with the contractile vacuole. There are small granules which group around the contractile vacuole. These do not take an osmic stain, but they are easily demonstrated by acid fuchsin (Fig. I, Plate).

The contractile vacuole of *Amœba proteus* does not take an osmic

stain; however, *Paramecium caudatum* occurs in the same slides and its contractile vacuoles are blackened by osmic acid as described by Nassonov (1925). Small vacuoles of variable sizes have been observed to be attached to the spheres with dark rims (Figs. 1–2, Plate). These are also noticed in the endoplasm; they are numerous around the contractile vacuole (Figs. 1–2, Plate). This causes the writer to believe that these small vacuoles originate from small granules which move about in the endoplasm and group around the contractile vacuole. They evidently flow together to form the new vacuole after the systole. The formation of the contractile vacuole is different therefore, from *Paramecium caudatum*, where the contractile vacuoles with their feeding canals are more or less permanent structures. The black granules are of variable sizes, and it is possible that these grow in size to form the spheres with black rims. The reaction of the acid fuchsin-thionin-aurantia and osmic acid procedures indicate that both types of bodies are Golgi material (Figs. 1–2, Plate). Also these bodies are similar to those described by Hirschler (1914), King and Gatenby (1923), Joyet-Lavergne (1926) and Hall (1930).

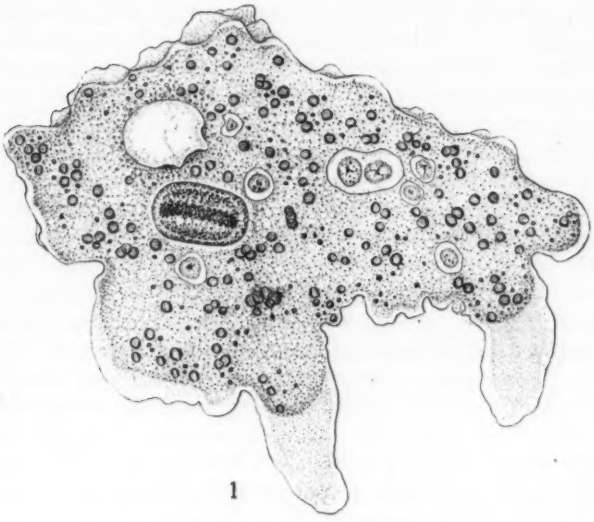
Bowen's modification of the acid fuchsin-thionin-aurantia method of procedure stains the Golgi apparatus of *Amœba proteus* blue. The spheres have dark blue rims (Fig. 1, Plate), whereas their centers are light blue. The Mann-Kopsch procedure stains them black; the large ones have light centers with black rims (Fig. 2, Plate).

Discussion

Hirschler (1914) described the Golgi apparatus (*i.e.*, spherules) of *Monocystis ascidæ* as rings and crescents. These have dark rims with centers. King and Gatenby (1923) described similar bodies as occurring in *Adelia*. They declared that these crescents and bead-like structures were comparable to the dictyosomes of metazoan cells. They also stated that these dictyosomes divide like those of the metazoan cells.

Hirschler (1914) found that the Golgi material of the Protozoa was made up of two substances, one of which takes a light stain, the other a dark one. Such a staining reaction occurs in the Golgi apparatus of *Amœba proteus*. One portion is chromophilic and the other chromophobic. This probably accounts for the fact that the spherules have dark rims. However, the writer does not find that the chromophobic portion has an affinity for acid fuchsin.

It is probably of interest to remark here that Vonwiller (1913) finds that neutral red used as a vital stain on *Amœba* colors the crystal-vacuoles (vacuoles which contain the characteristic crystals of *Amœba*)



1

2

Explanation of Plate

Both figures were drawn with the aid of an Abbé camera lucida. The chondriosomes were omitted in the drawings in order to avoid confusion.

FIG. 1. *Amœba proteus* fixed in Champy's solution and stained with acid fuchsin-thionin-aurantia. The Golgi bodies occur in clumps. The large globules have clear centers and dark rims. This gives the appearance of rings and crescent-shaped structures. The small globules are dark blue.

FIG. 2. *Amœba proteus* fixed and stained by Bowen's modification of Mann-Kopsch procedure. The Golgi bodies have a tendency to flow out into pseudopodia.

orange, whereas the smaller granules stain red. Joyet-Lavergne (1926) finds that vital staining with neutral red brings out the Golgi apparatus in the gregarines. These bodies occur as rings, crescents and spherules with dark red rims. Hall (1929) finds that neutral red used as a vital stain demonstrates the Golgi apparatus in various Protozoa. Hall (1930) finds similar globular bodies in *Trichamœba*, which react to neutral red and osmic acid. Occasionally crescent-shaped structures are noticed. Hall (1930*b*) shows that these globules are stained selectively with neutral red and are the same bodies which are blackened by osmic acid and silver impregnation. He used a mixture of Janus green and neutral red to distinguish these globules from the mitochondria of *Trichamœba*. The writer finds similar globules and crescent-shaped structures in *Amœba proteus* (Figs. 1–2, Plate). These bodies are smaller than the "crystal vacuoles," as described by Vonwiller (1913), but they are probably the same globules which stain bright red. These bodies have a tendency to flow out into the pseudopodia as described by Vonwiller. Therefore these globular bodies and crescent-shaped structures are probably the Golgi apparatus of *Amœba proteus* (Figs. 1–2, Plate).

Bowen (1923–1929) has suggested that the Golgi apparatus has a secretory function. He finds that the Golgi apparatus of glandular tissue hypertrophies at the beginning of the secretory cycle; when it hypertrophies, spherical and globular bodies are formed. These globular bodies have clear centers and dark rims when impregnated with osmic acid and are similar in many respects to those found in the Protozoa. A true Golgi network is not found in *Amœba proteus*, and these crescents and the globules are similar to the globules described by Bowen (1926). The writer is led to believe that the Protozoa are cells where secretion is a constant process; therefore, the Golgi apparatus would naturally be expected to be hypertrophied and occur as globules.

It has been suggested by Bowen (1926) that the relation between the Golgi apparatus and the secretory granules is homologous to that existing between the Golgi apparatus and the developing acrosome of vertebrate sperm, and this latter phenomenon can be used as a basis for interpretating the phenomena in the gland cell. The writer believes that a similar relationship exists between the crescent-shaped Golgi bodies and the minute vacuoles which occur throughout the endoplasm of *Amœba proteus*. These minute vacuoles are often attached to these crescent-shaped bodies (Fig. 2, Plate). They also occur throughout the endoplasm, and similar vacuoles occur in large numbers around the contractile vacuole. These vacuoles break into the contractile vacuole when the systole occurs. Day (1927) finds that the

contractile vacuole of *Amœba proteus* arises from the fusion of small vacuoles which probably owe their origin to the fusion and coalescence of ultra-microscopic droplets of soluble katabolic waste which may include the water of osmosis. The minute vacuoles described above seem to be similar in all respects to those described by Day (1927). The vacuole of *Amœba* is not a permanent structure and is formed by the fusion of minute vacuoles; this differs from the contractile vacuoles of *Paramecium caudatum*, where two permanent contractile vacuoles occur and pulsate successively. The contractile vacuoles of *Paramecium caudatum* have long "feeding" canals, and these structures are blackened by osmic acid (Nassonov, 1925).

Paramecium occurs in my slides along with *Amœba proteus*, and the contractile vacuoles of *Paramecium* are blackened by osmic acid; whereas, the contractile vacuole of *Amœba proteus* is not stained. This may be due to the difference in the formation of these vacuoles in the two Protozoa.

General Summary

1. The Golgi apparatus of *Amœba proteus* is the characteristic protozoan type of globules and spherules with clear centers and dark rims. These spherules, from a central focus, appear to be crescent-shaped structures. Small black granules appear without black centers.

2. These Golgi bodies are readily blackened by osmic acid and stained with thionin.

3. At the beginning of the secretory cycle of metazoan gland cells, the Golgi apparatus hypertrophies and globules are formed; the globules of *Amœba proteus* are believed to be homologous to these structures.

4. Since the secretory cycle is a continuous process in *Amœba*, it is suggested that this is the reason for the absence of a Golgi network.

5. It is suggested that the minute vacuoles which occur in the endoplasm of *Amœba* are associated with the crescent-like Golgi bodies, a relationship which is similar to that existing between the metazoan Golgi apparatus and the secretory cycle.

6. The contractile vacuole of *Amœba* is formed by a union of these minute vacuoles. It is possible that this is the reason why the contractile vacuole of *Amœba* is not blackened by osmic acid like the contractile vacuoles of *Paramecium caudatum*.

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